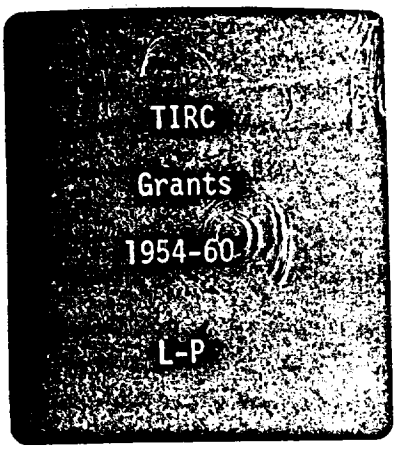

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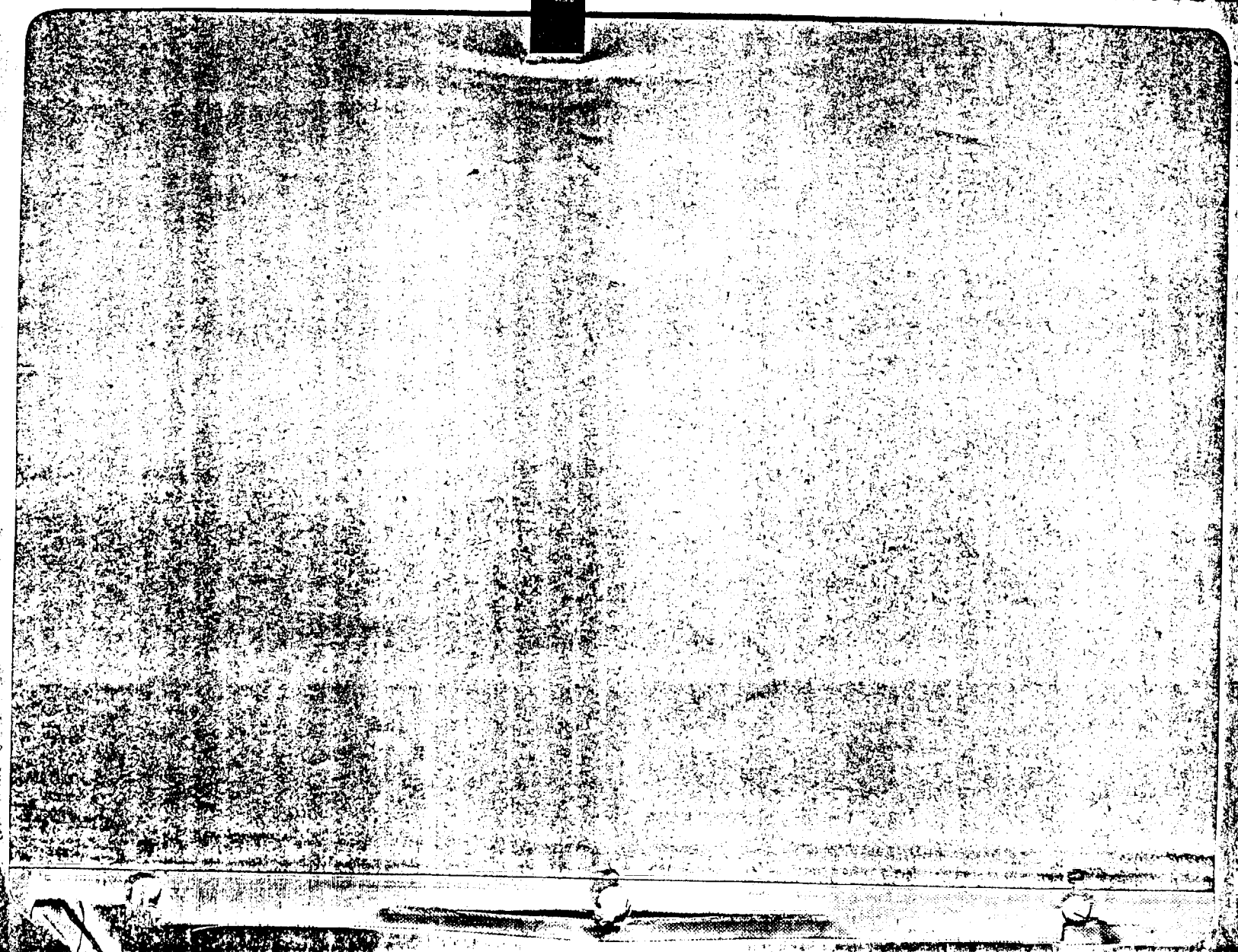

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Cf #501 #173

Activated Feb. 1, 1955
Renewed Feb. 1, 1956
Renewed Feb. 1, 1957

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

Date: July 18, 1957

1. Name of Investigator: *Thomas C. Laipply, M.D. and **William B. Wartman, M.D.

2. Title: *Professor of Pathology, Northwestern University Medical School and Chairman, Department of Laboratories, Chicago Wesley Memorial Hosp.
**Professor of Pathology and Chairman, Dept. of Pathology, Northwestern University Medical School

3. Institution & Address:
Northwestern University Medical School
303 East Chicago Avenue
Chicago 11, Illinois

4. Project or Subject: Significance of atypical epithelial hyperplasia and carcinoma in situ in relation of smoking, invasive bronchogenic carcinoma, and hormone therapy.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Detailed morphologic (gross and microscopic) examination of lungs from patients as follows:

GROUP I Lungs obtained at autopsies - from adults without bronchogenic carcinoma and without history of significant hormone therapy.

GROUP II Lungs, the seat of bronchogenic carcinoma obtained at autopsy and operations.

GROUP III Lungs obtained at autopsies from adults and children who have received hormone therapy.

It is estimated that in the period of investigation the number of cases in each group will be as follows:

GROUP I - 200 (100 cigarette smokers; 100 nonsmokers)
GROUP II - 75
GROUP III - 50

In all groups it is proposed that the tracheo-bronchial tree be studied by the method used by Auerbach et al as described in the New England Journal of Medicine, Vol. 256, page 97, January 17, 1957. Using this method 208 sections would be taken from the tracheo-bronchial tree from all cases in which the lungs are obtained at autopsy (Group I and III)

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5. (continued)

The number of sections taken from surgical specimens (Group II) would vary from about 25 to 208 sections depending on whether the specimens obtained were the result of lobectomies, pneumonectomies, or autopsies.

It is also proposed to inject the tracheo-bronchial tree with 10% formalin at the beginning of atuopsies prior to removal of organs so as to decrease the number of cases that are considered unsatisfactory because of lack of preserved epithelium.

Smoking histories will be obtained by mailing questionnaires to the closest relative deceased patients and from the patient directly after operation. The questionnaire with an accompanying letter (copies enclosed) have yielded a 88% response in the work on "Pathologic Anatomic Study of Human Lungs."

It is also hoped that the statistical "know-how" of the Tobacco Industry Research Committee could be available for analysis of results as was true in the recently completed study of lungs.

Each of the above three groups to be studied could be considered a separate project. Group I would require most (approximately 2/3) of the work and would provide data needed to corroborate or refute the recorded results of Auerbach, et al. The work on Groups II and III could be done simultaneously without additional laboratory facilities. Thus, it is proposed that all three groups of cases be studied over the same period of time.

It is hoped that regular meetings could be held with any investigators from different institutions engaged in similar studies. If the meetings are frequent enough to review problems and criteria of diagnosis it should be possible to combine the data for statistical analysis.

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6. Budget Plan:

Salaries		$23,200.00
Expendable Supplies		2,000.00
Permanent Equipment		1,600.00
Overhead (6%)		2,192.00
*Other	**	600.00
Total		$29,592.00

NOTE: *Travel allowance included because of possibility of meetings being held at institutions of different investigators. If, as in the past, meetings are held at and paid for by the Tobacco Industry Research Committee this should be omitted.

7. Anticipated Duration of Work:

3 years.

8. Facilities and Staff Available:

All those in Department of Pathology at Northwestern University Medical School and affiliated hospitals.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

NOTE: **As of June 30, 1957 $2,187.78 remaining in fund at Northwestern University from allotments granted for "Pathologic-Anatomic Study of Human Lungs" - deductable from total estimated budget of $29,592.00.

Recommended
Richard H. Young, M.D.
Dean, Medical School

Signature /s/ Thomas C. Laipply, M.D.
Director of Project

/s/ Arthur T. Schmehling
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

50(I) R2

Activated: 2/1/55
Renewed: 2/1/56

Application for Research Grant

Other

Total

Date: December 29, 1956

1. Name of Investigator:

Thomas C. Laigaly, M. D. and William B. Hartman, M.D.

2. Title:

Professor of Pathology, Northwestern University Medical School and Chairman, Department of Laboratories, Chicago Wesley Memorial Hospital.

3. Institution & Address:

Professor of Pathology and Chairman, Department of Pathology, Northwestern University Medical School.

Northwestern University Medical School
303 East Chicago Avenue
Chicago 11, Illinois

4. Project or Subject:

Pathologic anatomy study of cellular changes in human lungs.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Signature

Director

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Source: https://www.industrydocuments.ucsf.edu/docs/

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

6. Budget Plan:

Salaries	$2,000.00
Expendable Supplies	210.00
Permanent Equipment	
Overhead	
Other 8%	177.00
Total	$2,387.00

Date: December 23, 1946

7. Anticipated Duration of Work:

Thomas G. Laipply, M. D. and William E. Parham, M.D.

8. Facilities and Staff Available:

All those in Department of Pathology at Northwestern University Medical School and affiliated hospitals, viz: Hospital.

Department of Pediatrics and Children's Memorial Hospital

Northwestern University Medical School
303 East Chicago Avenue
Chicago 11, Illinois

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature
Director Thomas C. LAIPPLY, M. D.

Recommended
Richard H. Young, M. D.
Dean Northwestern Med. School.

For the Office of the Treasurer, Asst. Business Mngr. and Director of the Budget, 619 Clark St., Evanston, Illinois

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #223 (Cf. #128R2)

Report No. 6

Paul S. Larson
Medical College of Virginia

February 3, 1960

Enzymatic Transformations of Nicotine and Related Compounds

Dear Dr. Hockett:

In accordance with the suggestions in your recent letter occasion is taken to submit our application for renewal of the research grant for study of "Enzymatic Transformations of Nicotine and Related Compounds".

You will note that the application is made in the name of Dr. McKennis, whereas previous applications included myself, Dr. Haag and Dr. McKennis. This change was considered appropriate in view of the fact that Dr. McKennis, rather than Dr. Haag and myself, has carried the load of the program.

During the six-month period from July 1, 1959 to Dec. 31, 1959 means were developed for separating quaternary ammonium derivatives which arise as a result of the metabolism of nicotine. This has lead to the demonstration that, following administration of nicotine the dog excretes approximately 10 per cent of the dose in the form of a N'-methonium compound. Roughly one-half of the excreted compound has been isolated as a crystalline iodide which gives a satisfactory analysis and has properties in agreement with an authentic sample.

This biological methylation of the pyridine ring of nicotine has long been suspected, but never before demonstrated. In the light of previous literature on the biological properties of the compound we believe that the facts will eventually indicate that this reaction represents another detoxication (in the literal sense) mechanism available to the body for handling nicotine.

Preliminary data indicate also that three other N-methylpryidinium compounds arise as the result of nicotine metabolism. Two of these appear to be present in low concentration. Further work is required to show whether or not they are actually related to nicotine. A third, the methonium compound of cotinine, has been isolated from urine following administration of cotinine. The compound was obtained as a crystalline picrate and iodide, and identity was established by comparison with an authentic sample prepared by reaction of (-)-cotinine with methyl iodide. A paper dealing with the results of studies on methylation in the metabolism of nicotine has been accepted for presentation at the annual meeting of the Federation of American Societies for Experimental Biology in April 1960.

In addition to the foregoing, work was continued on the structure of the amphoteric nicotine metabolite which was obtained by chloroform extraction of alkalinized urine from dogs after nicotine administration. The structural assignment γ -(3-pyridyl)-γ -methylamino-β -oxo-N-methylbutyramide is now definite as a result of previous data and a recent Beckman rearrangement of the compound which afforded 3-pyridylacetic acid. An article by McKennis, Bowman and Turnbull describing the isolation and characterizations of the metabolite has been accepted by the Journal of the American Chemical Society. A copy of the manuscript is enclosed* together with a list of publications on the project to date.

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* Manuscript not received as yet.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

The past three and one-half years of studies with the generous support of the Tobacco Industry Research Committee have enabled the group here to separate and chemically verify the presence of nine compounds arising as a result of mammalian metabolism of nicotine. They probably account for about 40-50 per cent of excreted material. Preliminary biological studies have been made upon three metabolites, and improved or new procedures have been developed for the synthesis of seven of these metabolites.

We hope that future studies will be equally fruitful and continue to merit the support of the Committee.

Publications (TIRC supported project work)

1) Synthesis and properties of pyridylalanines, Herbert McKennis, Jr. and Edward R. Bowman, The Virginia Journal of Science, 8, 314 (1957).

2) Metabolism of γ -(3-pyridyl)-γ-oxobutyric acid, Lennox B. Turnbull, Edward R. Bowman and Herbert McKennis, Jr., Federation Proceedings, 17, 421 (1958).

3) γ -3-Pyridyl-γ-methylaminobutyric acid as a urinary metabolite of nicotine, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society, 79, 6342 (1957).

4) Metabolites of nicotine and a synthesis of nornicotine, Herbert McKennis, Jr., Lennox B. Turnbull, Harvey N. Wingfield, Jr. and Lovell J. Dewey, Journal of the American Chemical Society, 80, 1034 (1958).

5) The role of cotinine in nicotine metabolism, Herbert McKennis, Jr., L. B. Turnbull and E. R. Bowman, Abstracts of Communications, 14-37, IV International Congress of Biochemistry, Wien, 1-6 September 1958.

6) A constant rate infusion apparatus, Quentin S. McKennis, Edward R. Bowman and Herbert McKennis, Jr., Toxicology and Applied Pharmacology, 1, 61,(1959).

7) Metabolism of nicotine to γ-(3-pyridyl)-γ-methylaminobutyric acid, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society, 80, 6597 (1958).

8) Metabolism of nicotine in the human and excretion of pyridine compounds by smokers, Edward R. Bowman, Lennox B. Turnbull and Herbert McKennis, Jr., The Journal of Pharmacology and Experimental Therapeutics, 127, 92 (1959).

9) Demethylation of Cotinine in Vivo, Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman, and Einosuke Wada, Journal of the American Chemical Society, 81, 3951 (1959).

10) Oxidation of cotinine in vivo, Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr., Federation Proceedings, 18, 371 (1959).

11) Depressor Activity from Nicotine Metabolites, E. R. Bowman, H. B. Kennedy, Jr. E. Wada, and H. McKennis, Jr., Abstracts of Communications, XXI International Congress of Physiological Sciences, 41, Buenos Aires, 9-15 August 1959.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

12) The isolation and structure of a ketoamide formed in the metabolism of nicotine, Herbert McKennis, Jr., Edward R. Bowman, and Lennox B. Turnbull, Journal of the American Chemical Society, in press.

13) Methylation in the metabolism of (-)-nicotine, Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr., Federation Proceedings, in press.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#223

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-Second Street New York 17, N.Y.

Cf. #128
Activated 7/1/56
Renewed 7/1/57
Renewed 7/1/58

Application for Research Grant

Date: December 7, 1958

1. Name of Investigator: 1. Paul S. Larson, 2. H.B. Haag, 3. Herbert McKennis, Jr.

2. Title: 1. Professor of Pharmacology, 2. Professor of Pharmacology, 3. Professor of Pharmacology

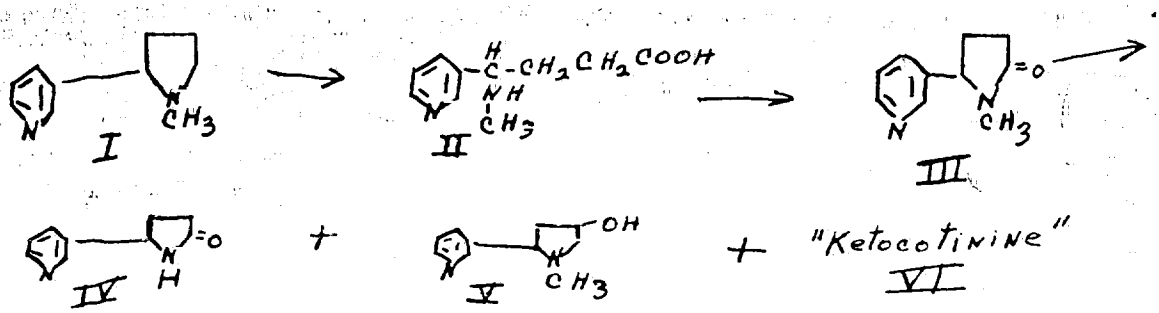
3. Institution & Address: Medical College of Virginia, Richmond 19, Virginia

4. Project or Subject: Enzymatic Transformations of Nicotine and Related Compounds

(Continuance application for period of July 1, 1959 - June 30, 1962.)

5. Detailed Plan of Procedure (use reverse side if additional space is needed):

During the past two and one-half years procedures for the isolation and synthesis of a variety of nicotine metabolites have been developed. In summary the following relationships have been established:



The foregoing compounds represent approximately 30% of the total metabolites of nicotine in the dog. y-3-Pyridyl-y-methylaminobutyric acid (II), cotinine (III), desmethylcotinine (IV) and hydroxycotinine (V) have all been isolated in pure condition and identified by comparison with synthetic compounds. With the exception of cotinine these are all new organic compounds for which synthetic methods were developed. These compounds have now been prepared in sufficient quantity to permit physiological studies. The syntheses provide also new total synthetic routes to both nornicotine and nicotine (I) and will allow investigators to selectively label nicotine, nornicotine or metabolites with isotopes for further study. Current evidence points to the occurrence of at least 15 Koenig positive metabolites

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of nicotine. An even larger number of Koenig negative metabolites may be revealed by use of procedures which have been developed to isolate the Koenig positive materials. Preliminary physiological studies with the isolated metabolites indicates that pressor activity is significantly or totally obliterated by the known biotransformation. y-3-Pyridyl-y-methylaminobutyric acid may, however, have an antidiuretic activity greater than that of nicotine (without the side effects). Nicotine metabolism in the human has been found to parallel (by chromatograms of the chloroform soluble fractions) that of the dog. Smokers excrete, however, Koenig positive material which may arise from non-nicotine pyridine components of smoke (see separate application).

It is proposed to study further nicotine metabolism, separate the metabolites chromatographically, identify the metabolites, and make possible physiological studies of the metabolites. Attention will be given to the possible reversible nature of reactions leading in vivo to the lactam metabolites, desmethylcotinine, hydroxycotinine, and cotinine. The possibility of relating nicotine intake to specific metabolites in the urine will also be considered. Two and one-half years of TIRC supported work has given rise to the publications given on the attached sheet.

6. Budget Plan:

Social Security	$ 240
Salaries	$17,900
Expendable Supplies	$ 4,500
Permanent Equipment	$ 6,500
Overhead (10%)	$ 3,003
Other Travel	$ 890
Total	$33,033

7. Anticipated Duration of Work: Three years (renewal of present three-year period which expires June 30, 1959). Progress will be reported at intervals and in the literature according to custom and past practice.

8. Facilities and Staff Available: General pharmacologic and biochemical equipment, Lardy type Warburg apparatus, refrigerator centrifuge, C^{14} counting equipment, nuclear instrument gas flow counter with automatic sample changer, paper chromatographic equipment, polarimeter, greenhouse facilities for growing C^{14} labelled plants, high pressure hydrogenation apparatus. Full time staff: Lennox B. Turnbull, Ph.D., M.S. to be hired. Part time includes: Drs. Herbert McKennis, Jr., Paul S. Larson, H.B. Haag, and E.R. Bowman, Public Health Research Fellow.

9. Additional Requirements: What, if any, will depend upon how the project develops.

10. Additional information (including relation of work to other projects and other sources of supply): The American Tobacco Company has provided C^{14} labelled nicotine for metabolic studies. E.R. Bowman has received a Public Health Research Fellowship from the National Heart Institute which will make possible his part-time participation in the project. He previously devoted full time and has already begun to train new workers in the necessary techniques.

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The breakdown for salaries is as follows:

Lennox B. Turnbull, Ph.D.		$ 8,500
To be filled	M.S.	$ 6,200
Technician part time	B.S.	$ 2,200
Animal man part time		$ 1,000
		$17,900

The breakdown on permanent equipment is as follows:

Gas chromatographic equipment	$ 4,200
X-Y recorder for autotitrator	$ 1,850
Partial cost of autotitrator	$ 450
	$ 6,500

Signature /s./ Paul S. Larson
Director of Project

/s./ B. B. Smith
Business Officer of the Institution

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Publications (TIRC supported project work):

1) Synthesis and properties of pyridylalanines, Herbert McKennis, Jr. and Edward R. Bowman, The Virginia Journal of Science, 8, 314 (1957).

2) Metabolism of y-(3-pyridyl)-y-oxobutyric acid, Lennox B. Turnbull, Edward R. Bowman and Herbert McKennis, Jr., Federation Proceedings, 17, 421 (1958).

3) y-3-Pyridyl-y-methylaminobutyric acid as a urinary metabolite of nicotine, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society, 79, 6342 (1957).

4) Metabolites of nicotine and a synthesis of nornicotine, Herbert McKennis, Jr., Lennox B. Turnbull, Harvey N. Wingfield, Jr. and Lovell J. Dewey, Journal of the American Chemical Society, 80, 1034 (1958).

5) The role of cotinine in nicotine metabolism, Herbert McKennis, Jr., L. B. Turnbull and E. R. Bowman, Abstracts of Communications, 14-37, IV International Congress of Biochemistry, Wien 1-6 September 1958.

6) A constant rate infusion apparatus, Quentin S. McKennis, Edward R. Bowman and Herbert McKennis, Jr., Toxicology and Applied Pharmacology, I, (1959) (in press).

7) Metabolism of nicotine to y-(3-pyridyl)-y-methylaminobutyric acid, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society, 80, (1958) (in press).

8) Metabolism of nicotine in the human and excretion of pyridine compounds by smokers, Edward R. Bowman, Lennox B. Turnbull and Herbert McKennis, Jr., Science (submitted for publication, November 26, 1958).

Four additional manuscripts have been partially completed.

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TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #128R2

Report No. 4

January 1, 1958-June 30, 1958

Paul S. Larson, Ph.D.
H. B. Haag, M.D.
Herbert Silvette, Ph.D.
Medical College of Virginia

Enzymatic Transformations of Nicotine

Summary. Studies on the metabolism of nicotine in the dog have been extended to show that cotinine, in addition to being a urinary metabolite, is also an intermediary metabolite which can be demethylated, possibly by transmethylation or oxidative demethylation, to give desmethylcotinine. Cotinine is also hydroxylated to give hydroxycotinine which has been isolated from the urine as the picrate salt of the acetyl derivative.

The human subject metabolized nicotine to cotinine. The latter has been isolated from the urine of a volunteer nonsmoker after ingestion of nicotine. Cotinine also appears in the urine of smokers along with a variety of other metabolites. It is considered that the excretion of pyridine compounds by the smoker will present a problem more complex than that of the non-smoker in view of the incompleteness of knowledge on the chemical composition of smoke.

Studies on the synthesis of hydroxycotinine have been made. A number of compounds related to the two metabolites, cotinine and y-3-(pyridyl)-y-methylaminobutyric acid have been prepared. Preliminary evidence is given to indicate the feasibility of two synthetic routes to hydroxycotinine.

The production of cotinine in vitro by the hydrogen peroxide-catalase system points to the likelihood that cotinine can arise in vivo by enzymatic action which is dependent upon catalase and hydrogen peroxide.

INTRODUCTION

Our semi-annual progress report (July 1, 1957 - December 31, 1957) described the synthesis of a number of compounds of interest in nicotine metabolism, the isolation of y-3-pyridyl-y-methylaminobutyric acid (III) and cotinine (II) from urine after administration of nicotine (I) to the dog and establishment of the metabolic degradation of nicotine:

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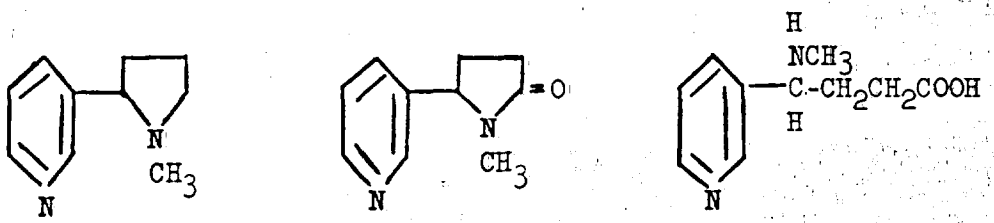

I II III

In addition to the foregoing preliminary data in support of the belief that cotinine played a key role in nicotine metabolism, pilot experiments on nicotine metabolism in the human were described.

The present report extends the observations which have previously been described, authenticates in part a hypothetical route of nicotine metabolism (cotinine ——> desmethylcotinine + hydroxycotinine), and provides an experimental basis for attributing to catalase an important enzymatic function in the degradation of nicotine in vivo.

For convenience of exposition this report is divided into the following sections:

I. Demethylation and Hydroxylation of Cotinine in vivo.

II. Metabolism of Nicotine in the Human.

III. The Urinary Excretion of Pyridine Compounds by Smokers.

IV. Hydrogen Peroxide-Catalase Oxidation of Nicotine.

V. Development of An Inexpensive Constant Rate Infusion Apparatus.

VI. Studies on The Synthesis of Derivatives of Cotinine.

VII. Publications and Papers Presented -- July 1, 1957-June 30, 1958.

VIII. Future Plans.

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II. METABOLISM OF NICOTINE IN THE HUMAN

Introduction

The previous semi-annual report describes preliminary studies in which a chloroform-extract of human urine was examined chromatographically

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and found to contain Koenig positive substances corresponding in Rf value to cotinine and "metabolite no. 3." These studies have been extended to facilitate a comparison with smokers' urine (section III) and unequivocal chemical identification of one of the metabolites present.

Experimental

A human subject (non-smoker) ingested 30 mg. of nicotine daily for three days in divided doses of 3 mg. per hour over a ten hour period on each day. The combined 24-hour samples of urine (total 5 liters) were extracted continuously with chloroform for twenty four hours. The residue from evaporation of the chloroform extract (567 mg.) upon paper chromatography (Whatman paper No. 1, ammonia, ethanol, butanol) yielded Koenig positive spots at Rf 0.61 and Rf 0.70. The former corresponded in Rf value to Koenig positive material obtained from urine of dogs following intravenous infusion of nicotine. The latter corresponded in Rf value to authentic cotinine. Upon chromatography (Whatman paper No. 1, sec-butanol, formic acid) Koenig positive spots at Rf 0.21 and 0.37 were obtained. Cochromatography showed the same correspondence with cotinine (Rf 0.37). This evidence seemed to suggest a resemblance between the metabolism of nicotine in the dog and in the human. In consequence chemical identification of cotinine was sought by an adaptation of the previously described methods. Control urine did not yield Koenig positive material.

The gummy material from the chloroform extraction was dissolved in 25 ml. of water and acidified to pH 2 with 5 N HCl. The solution was passed through a Dowex 50 (H^+) column. The column was washed with water until the effluent was neutral and then eluted with 80 ml. of N/1 ammonia water. The eluate was evaporated to dryness and then dissolved in 10 ml. of water and adjusted to pH 9 with N/1 ammonia water. The resulting solution was placed on a Dowex 1 ($O\bar{H}$) column (12 x 70 mm.). The effluent and water wash were combined and evaporated to dryness. The residue was treated with 25 ml. of chloroform and placed on a column of acid-washed alumina (5 g.) and eluted with ether containing 5-25 per cent methanol. The fractions yielding a single Koenig positive zone at Rf 0.72 (ammonia system) were combined and evaporated. The residue (10 mg.) of yellow oil yielded a crystalline picrate upon treatment with an excess of methanolic picric acid. The product was recrystallized to a constant micromelting point (100 - 102°) which was identical to that of an authentic sample of the picric acid salt of (-) cotinine. The mixed melting point showed no depression, and the infrared absorption spectra of authentic and metabolic products were identical.

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Summary and Conclusion

Since normal urine of the non-smoker does not yield Koenig positive material when treated in the foregoing procedure it is established that cotinine arises from the metabolism of nicotine in the human.

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III. THE URINARY EXCRETION OF PYRIDINE COMPOUNDS BY SMOKERS

Pooled urine from male human smokers (60 l) was collected, in the presence of toluene or chloroform and sodium flouride as preservative, as voluntary daytime contributions. The urine was adjusted to pH 9-10 by an addition of ammonia water and then extracted continuously with chloroform. The residue from the chloroform extract (7.09 g.) was treated with 40 ml. of water. The aqueous solution was separated from a residue. The latter was treated with 40 ml. of methanol which was separated from a residue. That the latter was devoid of Koenig positive material was demonstrated by paper chromatography of a chloroform extract. The methanolic solution, which contained Koenig positive material, was evaporated to dryness. The residue was dissolved in the aqueous extract. The solution was acidified to pH 2 with HCl and placed on a Dowex 50 (H+) column (2.5 cm. x 15 cm.). The column was then washed with 700 ml. of water, and finally eluted with 500 ml. of N/1 ammonia water until all of the Koenig positive material was removed. The eluate contained Koenig positive material: Rf 0.61, 0.75, 0.85 and 0.90 (minor) when chromatographed in the ammonia-ethanol-butanol system. These zones corresponded in Rf value to zones present in the original aqueous and methanolic extracts of the chloroform extracts of urine.

The ammoniacal solution from the Dowex 50 column was passed through Dowex 1 ($O\bar{H}$) (2.5 x 15 cm.). The effluent and water wash (1 l.) was concentrated to a yellow syrup (1.1 g.). The column was then eluted with 200 ml. of 1 m acetic acid. The acetic acid solution was concentrated to a brown syrup (459 mg.). The latter was dissolved in 20 ml. of absolute ethanol and treated with decolorizing carbon. The filtrate was evaporated to dryness. The brown residue (284 mg.) which showed a single Koenig positive zone at Rf 0.73 (ammonia-ethanol-butanol) was saved for further investigation.

The basic material (1. 1 g.) which was collected as effluent from the Dowex 1 ($O\bar{H}$) was dissolved in 25 ml. of chloroform and placed on a column of alumina (30 g.). The column was eluted with ether and methanolic ether. The fractions from the 0-10% methanolic ether contained Koenig positive material at Rf 0.71 and Rf 0.77 (ammonia-ethanol-butanol). A further fraction of Koenig positive material at Rf 0.61 was also obtained.

Those fractions with Rf 0.71 and Rf 0.77 concentrated to yield 668.5 mg. of white solid. By rechromatography with 0-5% methanolic ether the two concentrates were separated to give 150 mg. of crystalline material with Rf 0.77 and λ max 272 mu in ethanolic HCl. This will receive further study.

The fractions containing the Koenig positive material at Rf 0.71 were combined and concentrated to a brown gum (87 mg.). By cochromatography

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

with cotinine it was inferred that cotinine was present. Confirmation was achieved by conversion to cotinine picrate (m.p. 100-102°) which did not depress the melting point of authentic material.

The fractions with Rf 0.61 upon concentration yielded 118 mg. of brown gum. The material cochromatographed on paper with the desmethylcotinine-"hydroxycotinine" fraction obtained from nicotine metabolism in dogs. In consequence this material was treated with acetic anhydride and pyridine. The residue from concentration of this mixture was treated with methanol and reconcentrated to give 143 mg. of brown oil. This oil was dissolved in 5 ml. of chloroform and placed on a column of alumina (5 g.). The column was developed with methanolic ether (0-100%). To give Koenig positive material at Rf 0.75 and Rf 0.64 which were further investigated.

The Rf 0.75 fraction (80 mg.) consisted of an oil which was chromatographically identical on paper with acetylated hydroxycotinine which was previously isolated from the urine of dogs after nicotine infusion.

The Rf 0.64 fraction (17 mg.) was chromatographically identical with desmethylcotinine.

In addition to the foregoing survey of chloroform-soluble constituents of smokers' urine a preliminary survey of the substances not soluble in chloroform has been conducted. Both the aqueous and chloroform fractions present an experimental situation which is far more complicated than the corresponding situation observed following administration of nicotine to either the human or the dog.

For the moment no single explanation may be offered for the observed differences between the excretion of Koenig positive materials by the smoker and nicotine-treated subject. It is reasonable to suppose that some of the differences may arise as a result of absorption by the smoker of pyridine compounds other than nicotine. These substances may be metabolized to give a considerable variety of compounds. A further possibility is that the additional pyridine compounds may inhibit nicotine metabolism at some stage and thus give rise to new excretion products. Some of these factors will be considered in future studies.

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IV. HYDROGEN PEROXIDE-CATALASE OXIDATION OF NICOTINE

During the past few years emphasis has been given to methods for the isolation, characterization and identification of metabolites of nicotine. This work has made possible current investigations in which the physiological activity of the metabolites are being studied. In addition, the

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

variety of nicotine metabolites now available makes possible both theoretical and applied studies of the role of specific enzymes in the production of metabolites.

Present and previous reports present data which favors the belief that cotinine plays a key role in the metabolism of nicotine. Pilot experiments have, therefore, been conducted to establish the possible enzymatic route to cotinine.

Since cotinine has been observed as the result of autoxication of nicotine, it was considered that the formation of cotinine in vivo might arise as a result of the action of hydrogen peroxide-catalase action on nicotine. Keilin and Hartree have reported that ethanol is oxidized to acetaldehyde in vitro in the presence of hydrogen peroxide in phosphate buffer and that yields of the acetaldehyde are enhanced when the reaction is carried out in the presence of catalase. In our studies nicotine was treated with hydrogen peroxide and catalase under conditions similar to those employed by Keilin and Hartree for ethanol. Following the oxidative procedure the reaction mixture was steam distilled under alkaline conditions to remove unreacted nicotine. Aliquots of the solution were then extracted with chloroform. The chloroform solution was chromatographed on Whatman No. 1 with ammonia-ethanol-butanol. The single Koenig positive zone on the chromatogram corresponded in Rf value to that obtained from an authentic sample of cotinine. This experiment does not unequivocally establish the involvement of catalase in the oxidation of nicotine in vivo. It does provide, however, material evidence on which to develop further experimental studies.

V. DEVELOPMENT OF AN INEXPENSIVE CONSTANT RATE INFUSION APPARATUS

Introduction

In a variety of pharmacological and chemical studies there is a requirement for a device which will permit the infusion of solutions at a constant rate to an organism or chemical mixture. In simple chemical reactions there are a variety of devices for proportionate feed or constant rate injection of solutions can be accomplished by manual means, which are often tedious and inaccurate, or with the aid of positive drive mechanisms which are generally expensive, although under ideal conditions trouble-free and accurate. Apparatus in the latter category is usually elaborate and, in cases where simultaneous multiple tests are conducted, the cost of the apparatus can well exceed several thousand dollars.

In the course of studies in this laboratory where dogs were given aqueous solutions of nicotine intravenously over an eight-hour period it was desirable to develop moderately-priced apparatus for simultaneous

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

infusion at constant rate to six or more animals. By the advantageous selection of inexpensive commercial components such an apparatus was developed. So far as we are aware, no equivalent device has been described. The wide range of applicability of this simple apparatus prompts a report of it.

Experimental and Discussion

Dogs under pentobarbital anesthesia were cannulated at the femoral vein. The cannulae were attached by polyethylene tubing to a 25 or 50 ml. burette, equipped with solenoid valve (Houston Glass Fabricating Company, 5313 Harrison Boulevard, P.O. Box 9032, Houston 11, Texas, Cat. No. 824-2). The burette was enlarged at the top by means of a 60 mm. powder funnel which was connected by a short length of tubing to the top of the burette. In trial runs for adjustment a non-toxic solution such as saline was used to fill the burette. The solenoid valve was actuated by means of a repeat cycle timer (Andrew Technical Supply, 6972 North Clark Street, Chicago 26, Illinois). For the purposes of the study a delivery of one ml. in each 60 second period was selected. The timer (normally open) was set to close for one second in each minute.

After initial adjustment by a screw clamp to the required rate of flow, which is usually easily achieved empirically within the first 5 ml. of the burette by observing the initial rate and subsequent loss of rate (approximately 0.1 ml. with decrease of hydrostatic head corresponding to -1 ml. on the burette), the burette and reservoir are filled and positioned. The apparatus will infuse solutions intravenously to individual animals over 80 to 325 minute periods with an accuracy in the range of 10 per cent. Venous pressure is sufficiently constant to allow even flow from the time of the initial adjustment.

Since the apparatus is all glass, with the exception of the indicated organic material which can be readily converted to glass, corrosion does not become a problem. Tolerances are wide enough to insure ready assembly from ordinary parts, but some attention must be given to the diameter and wettability of the tube leading from the reservoir into the funnel to insure constant filling of the funnel.

If attention is given to stable placement of the cannula and elimination of foreign particles from the solutions no difficulties are ordinarily encountered. Grit and large particulate matter if present will prevent the solenoid valve from seating properly, and bring about an early emptying of the reservoir.

The total cost of special components for one animal is approximately forty dollars. Six or more additional animals may be simultaneously

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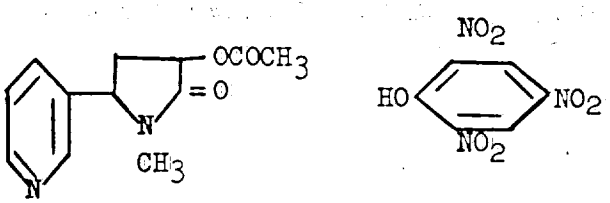
infused by the same timer at an approximate cost of fourteen dollars each. Additional economy can be effected by use of a surplus one r.p.m. motor mounted to actuate a microswitch by means of a heart cam or cam wheel.

Summary

An apparatus for infusion at constant rate, readily assembled from inexpensive commercial components has been described.

VI. STUDIES ON THE SYNTHESIS OF DERIVATIVES OF COTININE

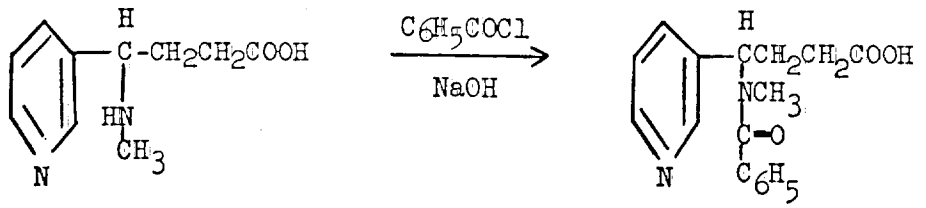
Following the administration of cotinine the urine of dogs contains a substance which, after chromatographic partial purification, can be acetylated with acetic anhydride-pyridine to yield an oil. The latter forms a crystalline picrate which has an empirical formula consistent with the following structural formula:



Preliminary data is sufficient to suggest that the metabolite "hydroxy-cotinine" has an OH group which is α to the carbonyl group of the pyrrolidone ring.

Based on the foregoing, several synthetic routes to the metabolic compound have been explored. Two routes now show promise and are now summarized to indicate the steps which have been carried out (a more detailed report will follow):

1.



(I) (II)

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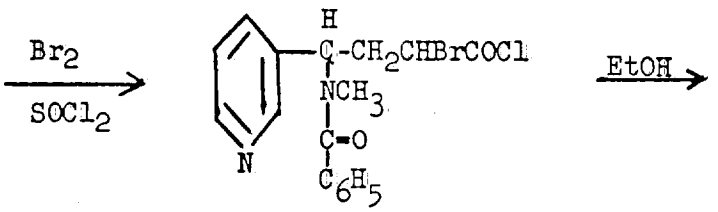
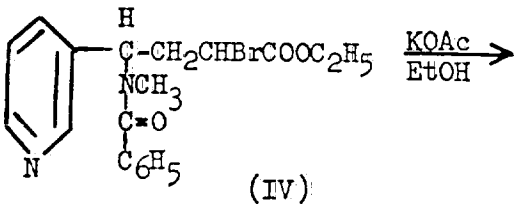
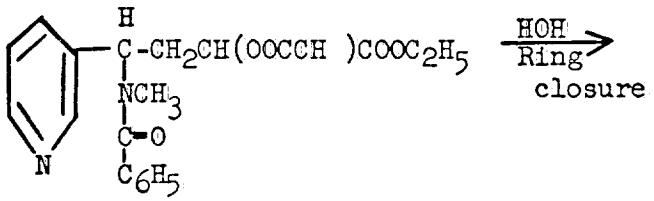


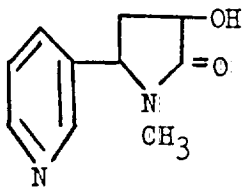
(III)



(IV)

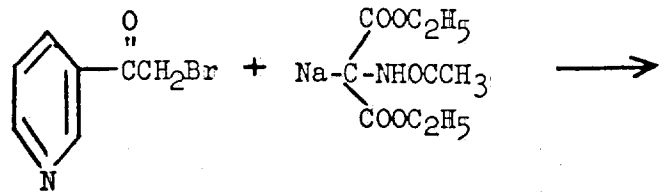


(V)



(VI)

2.



(I) (II)

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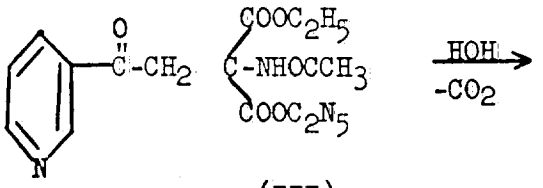

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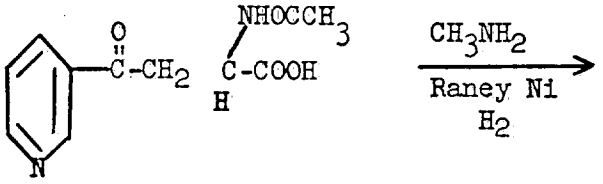
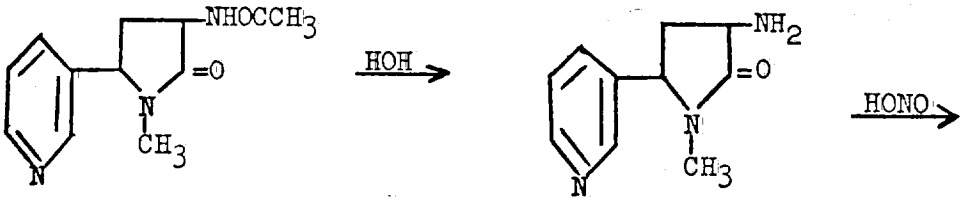
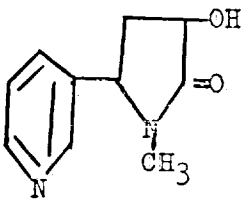


(IV)



(V) (VI)



(VII)

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In synthesis 1 intermediate (II) was prepared in good yield. The subsequent steps have yielded compounds which although not isolated in pure form appeared to be adequately pure to carry out the sequence. The final product (VI) hydroxycotinine although impure had chromatographic properties consistent with natural metabolic "hydroxycotinine."

In synthesis 2 products through (VI) have been obtained in good yield. Analytical data is not yet complete. Treatment of "aminocotinine" (VI) with nitrous acid afforded a product which cochromatographed with natural "hydroxycotinine."

Since both of the foregoing syntheses might be expected to yield a mixture of diastereoisomers in contrast to the single isomeric form expected in metabolism, steps are now being taken to prepare sufficient quantities of the materials to effect a separation of isomers for further chemical and biological studies.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

VII. PUBLICATIONS AND PAPERS -- JULY 1, 1957 - June 30, 1958

Publications

1) Synthesis and Properties of Pyridylalanines, Herbert McKennis, Jr., and Edward R. Bowman, The Virginia Journal of Science 8, 314 (1957).

2) Gamma-(3-Pyridyl)-Gamma-Methylaminobutyric Acid as a Urinary Metabolite of Nicotine, Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman, Journal of the American Chemical Society 79, 6342 (1957).

3) Metabolites of Nicotine and a Synthesis of Normicotine, Herbert McKennis, Jr., Lennox B. Turnbull, Harvey N. Wingfield, Jr., and Lovell J. Dewey, Journal of the American Chemical Society 80, 1634 (1958).

4) Metabolism of y-(3-Pyridyl)-y-Oxobutyric Acid, Lennox B. Turnbull, Edward R. Bowman and Herbert McKennis, Jr., Federation Proceedings 17, 421 (1958).

Papers Presented

1) Metabolic Fate of the Pyrollidine Ring of Nicotine, Herbert McKennis, Jr., Lennox B. Turnbull, and Edward R. Bowman, Southeastern Regional Meeting, American Chemical Society, Durham, N.C., Nov. 14, 1957.

2) New Metabolites of Nicotine, Lennox B. Turnbull, Herbert McKennis, Jr., and Edward R. Bowman, Biological Seminar, Medical College of Virginia, March 5, 1958.

3) Metabolism of y-(3-Pyridyl)-y-Oxobutyric Acid, Lennox B. Turnbull, Edward R. Bowman, and Herbert McKennis, Jr., 48th Annual Meeting, American Society for Pharmacology and Experimental Therapeutics, Philadelphia, Penna., April 16, 1958.

4) Metabolism of Nicotine in the Human, Edward R. Bowman, Lennox B. Turnbull, and Herbert McKennis, Jr., Annual Meeting, The Virginia Academy of Science, Roanoke, Va., May 9, 1958.

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VIII. Future Plans

A continuation of studies on the synthesis of hydroxycotinine and related compounds is contemplated. This will facilitate chemical characterization of urinary metabolites and aid in the interpretation of enzymatic studies on the degradation of nicotine. A parallel study of nornicotine metabolism is being inaugurated to facilitate interpretation of the urinary pyridine compounds of the smoker and to assist in the interpretation of nicotine metabolism.

Since the current program is now entering the final year of the three year period under support of the Tobacco Industry Research Committee it is planned to formulate a reapplication for support in the coming fall.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Cardiovascular, pharmacology and chemistry
Committee:
Dr. Jacobson, Chairman
Dr. Cattell
Dr. Bing

#286

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

Application for Research Grant

Date: July 20, 1960

1. Name of Investigator: Paul S. Larson, Ph.D.

2. Title: Professor of Pharmacology

3. Institution & Address: Medical College of Virginia
Richmond 19, Virginia

4. Project or Subject: The effect of tobacco smoke and nicotine on ascorbic acid metabolism.

5. Detailed Plan of Procedure:

Numerous reports have included claims for an effect of tobacco smoke and specifically nicotine on ascorbic acid metabolism. Representative of these, and some to the contrary, are the following.

Decreased urinary excretion of ascorbic acid in smokers (Strauss & Scheer, Zschr. Vitamin-forsch. 9:39, 1939; Goyanna, Brasil med. 69:173, 1955). Increased followed by decreased excretion in the urine in mice exposed to cigarette smoke for 20 minutes a day (Venulet & Moskwa, Polski Tygod, Lekarski 7:281, 1952).

Decreased ascorbic acid content in the milk of smoking mothers (Venulet & Danysz, Acta Physiol. Polonica 4:646, 1954; Pediat. Polska 30:811, 1955).

Lowered blood ascorbic acid levels in smokers or from smoking tests (Harmsen, Deut, med. Wschr. 67, 790, 1941; Venulet & Moskwa, Polski Tygod. Lekarski, 7:281, 1952; Bourquin & Musmanno, Am. J. Digest. Dis. 20:75, 1953). However, Hogler (Wien. med. Wschr. 93:309, 1943) in studies on more than 100 smokers and non-smokers found no regular differences in fasting ascorbic acid blood-levels, even in acute tests (smoking 3 cigarettes in succession on an empty stomach). Also, Wenzel and Beckloff (J. Am. Pharmaceut. Assoc., Sci. Ed. 47:338, 1958) did not find significant differences in the serum ascorbic acid levels of rabbits maintained on control diet or fed 0.1% cholesterol in the diet, or given 2.8 mg./kg./day nicotine in the drinking water, or given combined cholesterol and nicotine.

Decreased adrenal ascorbic acid content following nicotine administration (rat: Maren, Proc. Soc. Exp. Biol. Med. 77:521, 1951). Decreased adrenal ascorbic acid content following exposure to tobacco smoke (frog: Venulet & Moskwa, Polski Tygod, Lekarski 7:281, 1952; mouse: Venulet & Majcherski, ibid 10:6, 1955; guinea pig and rabbit: Lupu, Velican & Mihaescu, Session of Med. Sci. Section of Roumanian Acad. of 22-24 Jan. 1954, p. 141-168).

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In in vitro studies, Reif (Biochem. Z. 315:310, 1943) found that destruction of ascorbic acid by nicotine proceeded only slowly in pure distilled water but could be markedly accelerated by small amounts of copper. With small amounts of nicotine the reaction stopped at the dehydroascorbic acid stage; with large amounts, no ascorbic acid was recoverable by treatment with hydrogen sulfide. Bourquin & Musmanno (1953 reference above) added to blood of known ascorbic acid content, nicotine in amount estimated to equal the amount which might be absorbed in heavy smoking and in 3 experiments found that 24.4 - 31.6% of the original ascorbic acid had been lost after 5 or 10 minutes.

If this effect indeed occurs, it would seem well to learn more concerning its magnitude and its mechanism, so as to help judge its possible physiological importance to the body economy, since the vitamin has been implicated in steroid and cholesterol synthesis, iron metabolism, the degradation of tyrosine, collagen formation, cellular respirations, and as a factor in the bodies resistance to stress.

Proposed Research

Analysis of 24-hour urine samples of large groups of smoking and nonsmoking students and volunteers using the colorimetric method of Roe and Kuether (J. Biol. Chem. 147:399, 1943) which measures both forms of the vitamin by the color produced by reaction with 2,4-dinitrophenyl-hydrazine after oxidizing the reduced form by treatment with "Norit" and acetic acid. The assay commonly used by the workers cited above was the titrimetric decolorization of the dye, 2,6-dichlorophenol indophenol by the reducing action of l-ascorbic acid. This does not account for the oxidized form (which also has antiscorbutic activity) unless first reduction with hydrogen sulfide is effected, a precaution mentioned by only one of the investigators cited (Reif).

Observations on ascorbic acid excretion in "ascorbic acid loaded" volunteers during period of abstinence, smoking regular and possibly denicotinized cigarettes, and in nonsmokers.

Animal studies using both species that can synthesize ascorbic acid and ones that cannot. Particular emphasis will be placed on the use of C^{14} labeled ascorbic acid to follow the excretion of C^{14} as well as ascorbic acid, with and without exposure to nicotine, as well as preparing autoradiograms of urine following chromatographic development in suitable solvent systems.

In vitro studies on ascorbic acid and nicotine combinations in aqueous and blood serum media using both cold and C^{14} ascorbic acid. Rate of disappearance of ascorbic acid will be followed and autoradiograms will help determine the formation of chemical transformations.

Further direction will depend on findings during the course of the above studies.

6. Budget Plan: 1st year

Salaries	2,550.
Expendable Supplies	2,000.
Permanent Equipment	--
Overhead (10%)	470.
Other (Travel)	150.
Total	$5,170.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

7. Anticipated Duration of Work: Two years (The second year budget should be about the same as the first year one.

8. Facilities and Staff Available:

Facilities include spectrophotomer, chromatographic and C^{14} counting equipment needed for the study. Mr. Robert I. Toborowsky will spend virtually full time on the project. Mr. Toborowsky is a graduate student in his second year of study for the Ph.D. degree. He is an outstanding student with a straight "A" record and the study will be his thesis research. $2,350 of the salary item is to be for his fellowship stipend ($2,000) and tuition costs ($350); the remaining $200 is to contribute toward animal technicians salaries. Dr. Larson and the other members of our staff will contribute in supervisory capacities.

9. Additional Requirements: None

10. Additional Information:

The possibility exists that relationships to nicotine metabolism studies in progress will develop from this study.

Signature Paul S. Larson
Director of Project

R. Blackwell Smith Jr.
President

10035541356

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#128R2

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

Application For Research Grant

Activated 7/1/56
Renewed 7/1/57

Date: January 24, 1958

1. Name of Investigator:
   1. Paul S. Larson
   2. Harvey B. Haag
   3. Herbert McKennis, Jr.
2. Title:
   1. Professor of Pharmacology
   2. Professor of Pharmacology
   3. Professor of Pharmacology
3. Institution & Address:
   Medical College of Virginia
   Richmond 19, Virginia
4. Project or Subject:
   Enzymatic Transformation of Nicotine and Related Compounds.

100354135?

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

In the enclosed semi-annual progress report it will be seen that by means of fractional crystalization, chromatography and extraction it has been possible to identify two metabolites of nicotine and separate others. By use of these methods it is planned to investigate further the nature of the unidentified nicotine metabolites and to chemically identify these. Studies are also planned which will provide information about nicotine metabolites which do not give positive Koenig tests. It is considered that experiments with C-14 or tritium labelled nicotine will give the most satisfactory results in the search for the Koenig negative compounds. Current experiments have shown that the major portion of nicotine is metabolized to Koenig negative compounds which contain either a substituted or degraded pyridine nucleus. The radioactive compounds should, in addition to providing a satisfactory route to the uninvestigated fraction of metabolized nicotine, facilitate greatly studies on the role of the bile in excretion of nicotine and metabolites.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Social Security	230
Salaries	16,400
Expendable Supplies	4,500
Permanent Equipment	3,435
Overhead (10%)	2,572
Other	1,100
Total	28,237

7. Anticipated Duration of Work: One year; it is intended to review the progress of the current investigation in the fall of 1958 and thereafter to make an application for extension of the grant.

8. Facilities and Staff Available:

General pharmacologic and biochemical equipment; fraction collectors, Lardy type Warburg apparatus, Refrigerated centrifuge, C^{14} counting equipment (Libby anti-coincidence counter, nuclear instrument glass flow counter with automatic sample changer) paper chromatographic equipment, polarimeter, greenhouse facilities for growing C^{14} labelled plants.

9. Additional Requirements: What if any, will depend upon how the project develops.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Salary breakdown:	Lennox B. Turnbull, Ph.D.	$8,400	
	Edward R. Bowman, M.S.	6,200	
	Lab. Assistant (part-time)	1,200	
	Animal man (part-time)	600	
	Social Security	230	
Other-breakdown:	Travel	$750	
Fees for human volunteers (nicotine)		350	
Permanent equipment-breakdown:	Spinco Preparative Centrifuge (with rotors)		$2950
	Constant infusion pump		535

The centrifuge will make possible preparation of active enzymatic fractions and the pump will insure reproductibility in animal experiments.

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/s/ Paul S. Larson
/s/ Harvey B. Haag
/s/ Herbert McKennis, Jr.

Signature
Director of Project

/s/ W. E. Hopkins
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Initiated 7/1/56

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

RENEWAL 128R1

Application For Research Grant

Date: January 24, 1957

1. Name of Investigator: 1. Paul S. Larson
2. H. B. Haag
3. Herbert McKennis, Jr.

2. Title: 1. Professor of Pharmacology
2. Professor of Pharmacology
3. Professor of Research Pharmacology

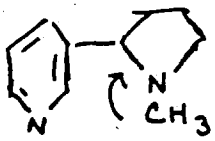
3. Institution & Address: Medical College of Virginia
Richmond 19, Virginia

4. Project or Subject:

Enzymatic Transformations of Nicotine and Related Compounds.
(Continuance application for period of July 1, 1957 - June 30, 1958)

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

In the enclosed half-yearly progress report it will be seen that the metabolism of nicotine has been considered to fall into three categories. Current work is concerned almost exclusively with the "cotinine pathway" of nicotine metabolism. Methods have been developed for the synthesis of compounds of interest for this pathway, and methods have been devised for the isolation and separation of the compounds. Currently work on the metabolism of the intermediates (actual and postulated) is in progress. It is anticipated that during the months that follow substantial further progress will have been made. It is then planned to investigate the nature of the physiological processes involved in attack of the pyrrolidine ring on the opposite side of the ring as indicated below:



While the ring cleavage currently being studied leads to the development of compounds which appear to be devoid of nicotine-like action, it can be seen that many of the products from ring attack and cleavage (as shown above) may resemble nicotine or perhaps some of the pressor amines in their action. Plans would include a systematic study of methods for isolation and identification of products which may be anticipated from this general type of biological degradation. Attempts will be made to correlate the occurrence of 3-pyridylpropyl ketone in fermented tobacco to metabolic pathways deemed capable of producing the same compound in higher organisms. The improved methods for chemical synthesis of nornicotine and nicotine which have arisen from work accomplished during the first six months of the current grant will make possible new applications of C^{14} to the study of nicotine metabolism as herein projected.

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Source: https://www.industrydocumentsucsf.edu/docs/tfvm0000

6. Budget Plan: (Second Year)

Social Security	$ 189.00
Salaries	13,700.00
Expendable Supplies	4,000.00
Permanent Equipment	2,800.00
* Overhead (10%)	2,103.90
Other (Travel)	350.00
Total	$23,142.90

7. Anticipated Duration of Work:

Two years

8. Facilities and Staff Available:

General pharmacologic and biochemical equipment. Lardy type Warburg apparatus, refrigerator centrifuge, C^{14} counting equipment (Libby anticoincedence counter, nuclear instrument gas flow counter with automatic sample changer) paper chromatographic equipment, polarimeter, green house facilities for growing C^{14} labelled plants.

Staff: full time: Lennox B. Turnbull, Ph.D., Edward R. Bowman, M.S.,
part time: Dr. Paul S. Larson, Dr. H. B. Haag, Dr. Herbert McKennis, Jr.

9. Additional Requirements:

What, if any, will depend upon how the project develops.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The breakdown of permanent equipment items is as follows:

2 Fraction Collector (with set of siphons)	$1,950.00
1 Unterzaucher oxygen determination apparatus	850.00

The fraction collectors are required to relieve personnel from the time-consuming manual collection of samples.
The oxygen determination apparatus is required to make possible determination of the oxygen content of some of the pyridine derivatives. The apparatus also can be used in determining the source and biochemical stability of oxygen intermediates (through use of O^{18}). Facilities for determination of oxygen are very sparsely distributed throughout the country and are not generally available for outside work.

The breakdown for salaries is as follows:

L. B. Turnbull, Ph.D.	$8,000.00
E. R. Bowman, M.S.	5,700.00

Signature Paul S. Larson
H. B. Haag
Herbert McKennis, Jr.
Director of Project

W. T. Tompkins
Business Officer of the Institution
Comptroller

100354360

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan:
Salaries
Expendable Supplies
Application For Research Grant
Overhead
Other

#128

Date: March 14, 1956

1. Name of Investigator: 1. Paul S. Larson, Ph. D. 2. H. B. Haag, M.D.
3. Herbert McKennis, Ph.D.

2. Title: 1. Professor of Pharmacology 2. Professor of Pharmacology
3. Professor of Research Pharmacology

3. Institution and Address: Medical College of Virginia
Richmond 19, Virginia

4. Project or Subject: Enzymatic Transformations of Nicotine

9. Additional Requirements:

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):
10. Additional information including relation of work to other projects and other sources of support:

Numerous experiments on animals have shown that only a small percent of administered nicotine is excreted unchanged (Larson, Ind. Eng. Chem. 44, 279, 1952). However, studies with C^{14} labeled nicotine show that virtually all of the C^{14} is excreted in the urine within 36 hours (Ganz, Kelsey and Gelling, J. Pharmacol. & Exper. Therap. 103, 209, 1951; Bennett, Tedeschi & Larson, Arch. int. pharmacodyn. 98, 221, 1954). Recent studies in this laboratory on urine from dogs receiving C^{14} labeled nicotine indicate the presence of three major and possibly four minor metabolites of nicotine (Owen & Larson, Fed. Proceedings 14, 376, 1955). The chemical nature of these biotransformation products is currently being investigated.

To complement these in vivo studies on nicotine metabolism, it is proposed to initiate in vitro studies directed toward elucidating the enzyme systems involved. Since early studies in which tissue slices were used, no investigations on the enzymatic transformation of nicotine by animals have been recorded (Werle et al., Biochem. Z. 308, 355, 1941; ibid, 318, 531, 1948; Annal. d. Chem. 562, 60, 1949). These studies provide little information other than a few characteristics of the systems involved.

Data on possible intermediates in the metabolism of nicotine being obtained in our laboratory, together with general advances in enzymology, make possible a study in systems less complex than whole tissue slices. For example, purified preparations fortified with co-factors according to modern enzymatic techniques should yield new information. The fact that possible intermediates have been disclosed also makes possible new studies on the effect of oxidation - reduction and other type enzymes on these compounds. Results from the enzymatic studies would be compared to intact animal studies using various species. C^{14} labeled nicotine will be used in experiments where such labeling is desirable.

/s/ [illegible] Comptroller
Business Officer of the Institution

1003541361

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	$13,000
Expendable Supplies	4,000
Permanent Equipment	10,050
Overhead	1,730
Other	300
Total	$29,080

*10% on all items excepting permanent equipment.

7. Anticipated Duration of Work: Three years

8. Facilities and Staff Available:

General pharmacologic and biochemical equipment and laboratory. Lardy-type Warburg apparatus, refrigerated centrifuge, C14 counting equipment (Libby anticoincidence counter, Nuclear Instruments gas-flow counter with automatic sample changer), paper chromatographic equipment and electrolytic desalter, greenhouse facilities for growing C14 labeled plants

Staff: Part-time services of - Dr. Paul S. Larson
Dr. H. B. Haag
Dr. Herbert McKennis

9. Additional Requirements:

What, if any, there may be will depend on how the project develops.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The breakdown of permanent equipment items is as follows: Perkin Elmer Model 137 Spectracord 4000 with attachments $8400, thermocap $96, circulating pump $42, Precision Senior Isotemp Oven $240, International Centrifuge Size No. 2 with attachments $960, Welch vacuum pump 1402 UEM with guard $310. The major item of the Spectracord is needed for reaction rate studies, indentification studies, etc., in this and in the other programs underway in this laboratory involving tobacco and its constituents.

The salary item is intended for stipends for two additional professional scientists to be added to the staff; one at the M.S. level ($5,500), the other at the Ph.D. level ($7,500).

In view of the need from the start of this project for the permanent equipment items, it is requested that if this application is granted that the initial payment will be for $15,000.

/s/ Paul S. Larson
/s/ H. B. Haag
/s/ Herbert McKennis Jr.
Signature of Director of Project

/s/ W. F. Tompkins, Comptroller
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

CROSS REFERENCE SHEET

Name or Subject: Paul S. Larson

Regarding: Research Grant #24

SEE

W. B. Haag

10035441363

Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

Committee:
Dr. Jacobson, Chm.
Dr. Comroe
Dr. Rienhoff

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-second Street
New York 17, N. Y.

#265

Application for Research Grant

Date: February 6, 1960

1. Name of Investigator: Gustave A. Laurenzi, M.D.

2. Title: Assistant Professor of Medicine
Coordinator of the Division of Respiratory Diseases

3. Institution & Address: Seton Hall College of Medicine
Medical Center
Jersey City 4, New Jersey

4. Project or Subject: Studies in bronchitis: A correlated investigation of the capacity of the lower respiratory tract to maintain sterility and the factors which interfere with the protective mechanisms involved. I. Human: A study of the bacterial flora of the upper and lower respiratory tract in normals (smokers and non-smokers) and chronic bronchitics (smokers and non-smokers). II. Animals: A study of the mechanisms whereby the respiratory tract clears bacteria: (a) demonstration of clearing; (b) demonstration (anatomical) of the sites of clearing and identification of the protective mechanisms involved, and (c) the study and comparison of the effects of cigarette smoke and sulfur dioxide on the clearing capacity.

1003541364

5. Detailed Plan of Procedure:
Headings refer to those in Section 4.
I. Human study: The examination of expectorated sputum for bacteria is misleading in that secretions from the lower respiratory tract are contaminated by oropharyngeal secretions. Studies have shown that in the normal, secretions in the bronchial tree below the carina are sterile. I am concerned with demonstrating this sterility by comparing the bacteriology of aspirates from the lower bronchial tree with the bacteriology of expectorated sputum and oropharyngeal secretions. I propose to study normal smokers and nonsmokers and patients with chronic bronchitis. Bronchial swabs and washings will be obtained under sterile conditions at bronchoscopy or by intubation with catheters (as in bronchography). All specimens are to be homogenized and plated out on appropriate media. (Please see section 10 for work done.)
II. Animal study: (a) In substance the method employed provides a means of administering quantitative doses of bacteria by aerosol to small animals for varying lengths of time and then observing what happens quantitatively to those bacteria that reach the lungs. Animals (rats, mice) are to be exposed to bacteria (pneumococci, staphylococci) in a large plexiglass cylinder. Broth cultures of the bacteria are atomized through a collision nebulizer. The cylinder acts as a mixing assembly and inhalation chamber. Groups of exposed animals are sacrificed at different time intervals after exposure; the lungs are removed aseptically and homogenized on a high speed tissue homogenizer; and bacterial counts are done on the homogenate by counting

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

R: REDACTED MATERIAL

colonies on agar pour plates. Time-concentration curves are constructed so that it is possible to predict within a range the number of bacteria present in an animal's lung at a certain time interval following the administration of a specific challenging dose. I am most interested in how fast the lung clears the bacteria, i.e. 1,000 organisms immediately after exposure; 0 organisms 24 hours later. (This year I have had the opportunity to work with this method following the intratracheal route of administration. By this route rats clear 40% to 50% of bacteria in 24 hours. Other work shows complete clearing of bacteria from the lungs of mice following aerosol administration.)

(b) The fluorescent-antibody technique is especially adaptable to identifying the major anatomical sites of clearing. In this method specific antibodies are made to a specific organism, and these antibodies are then conjugated with fluorescein isothiocyanate. A high titer fluorescein-antibody solution is employed as an immunochemical stain to sections of tissue. Examination of the tissue under the fluorescence microscope reveals the exact sites of penetration and phagocytosis. The identification of the protective mechanisms can be done by blocking the many known mechanical and immune factors. Ciliary action may be interfered with by anesthetics or alcohol; phagocytosis may be lessened by nitrogen mustards; humoral factors may be blocked by chemical means, i.e. blocking of complement by zymosan. The time-concentration curves will be worked out for animals under these various conditions, and comparisons will be made with the untreated animals.

(c) Different groups of animals will be exposed to cigarette smoke and sulfur dioxide. This will be done by administering bacteria in an aerosol with either smoke or sulfur dioxide and by giving bacteria to animals after more chronic exposure to these materials. Again, time-concentration curves will be determined as outlined in (a) and comparisons will be made.

6. Budget Plan:

Salaries	REDACTED
Expendable Supplies	1,944.00
Permanent Equipment	7,489.00
Overhead (15%)	2,525.00
Other	-
Total	REDACTED

10035541365

7. Anticipated Duration of Work: This grant request is for one year's support. The permanent equipment will make up an established aerosol laboratory. With the great versatility of the aerosol method I anticipate a much longer study of the many ramifications of this project.

8. Facilities and Staff Available: There will be adequate office and laboratory space at the medical school. There is ample space for the housing and care of laboratory animals, and culture media and basic bacteriological needs will be supplied by the hospital microbiology department. This project will require the part time participation of a pathologist and a thoracic surgeon. A bacteriology technician and a secretary will also be included.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

9. Additional Requirements: The use of aerosolized cultures of bacteria requires maximum protection for personnel. Concerning this matter I have visited and consulted the Bacterial Warfare Center at Fort Detrick, Maryland, and the School of Public Health and Department of Industrial Hygiene at Harvard. The plan of this experiment and the protective measures we will use meet with their approval.

10. Additional Information (Including relation of work to other projects and other sources of supply): This year I have studied 25 patients by the method described in section 5, human study. All of these patients were bronchoscoped because of the suspicion of bronchogenic carcinoma. Four of these patients had chronic bronchitis, and all of them were moderate to heavy cigarette smokers for 25 years. Bronchial aspirates from the 21 non-bronchitics and one bronchitic were sterile. This indicates that any effect that smoking may have on the bronchial tree does not interfere with the normal clearing of bacteria. (This material is being prepared for a preliminary report in the New England Journal of Medicine.) A study of the mechanisms whereby the lower respiratory tract is able to rid itself of bacteria and what factors upset these mechanisms so that bacteria are able to take hold, colonize, and cause bronchial infection is fundamental to the entire problem of chronic bronchitis. I am also seeking aid from the National Tuberculosis Association to continue a similar study on the intratracheal administration of bacteria to animals.

/s./ Gustave A. Laurenzi, M.D.
Director of Project

/s./ Joseph F. Salerno
Business Officer of the Institution

100354366

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

CURRICULUM VITAE

GUSTAVE A. LAURENZI

Undergraduate School	New York University, B.A., cum laude.	1949
Medical School	Georgetown University School of Medicine, M.D., magna cum laude.	1953
Pathology	Intern in pathology, Mallory Institute of Pathology, Boston, Massachusetts	July, 1953-June, 1954
Medicine	Intern in Medicine, Yale University Medical Service, Grace-New Haven Community Hospital, New Haven, Conn.	July, 1954-June, 1955
Medicine	Assistant resident in Medicine, First (Columbia) Medical Service, Bellevue Medical Center, New York 16, New York	July, 1955-June, 1956
Pulmonary Physiology	Research fellow, Cardiopulmonary Laboratory, Columbia-Presbyterian Medical Center, New York 32, New York U.S.P.H.S. Postdoctorate fellow. Assistant Physician to Presbyterian Hospital.	July, 1956-June 1957
Pulmonary Physiology	Research fellow (same as above). Fellow in Medicine of the National Foundation for Infantile Paralysis. Assistant Physician to Presbyterian Hospital.	July, 1957-June, 1958
Clinical Chest & Medicine	Chief resident physician, Chest Service, Bellevue Medical Center, New York 16, New York. Teaching Fellow of the National Tuberculosis Association.	July, 1958-June, 1959
Bacteriology & Immunology	Research Fellow of the National Tuberculosis Association. Channing Research Fellow in Bacteriology and Immunology at Harvard, Channing Laboratory, Mallory Institute of Pathology, Boston City Hospital, Boston, Mass. Clinical Assistant in Medicine, and Chest Consultant, Second & Fourth (Harvard) Medical Services, Boston City Hospital, Boston, Mass.	July, 1959-June, 1960
	Assistant Professor of Medicine Coordinator of Division of Respiratory Diseases, Seton Hall College of Medicine, Jersey City, New Jersey	July, 1960

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Committee:
Drs. Jacobson, Chm.
Kotin
Rienhoff

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

#265M
(M - modified)
(Cf. #265
considered on 3/11/60)

Application for Research Grant

Date: April 22, 1960

1. Name of Investigator: Gustave A. Laurenzi, M.D.

2. Title: Assistant Professor of Medicine
Coordinator of the Division of Respiratory Diseases

3. Institution & Address: Seton Hall College of Medicine
Medical Center
Jersey City 4, N.J.

4. Project or Subject: Studies in bronchitis: A correlated investigation of:
(a) the effect of smoking on the bacteriology of the respiratory tract of humans, and
(b) the effect of cigarette smoke and sulfur dioxide (air pollutant) on the clearing of bacteria from the respiratory tracts of small animals (mice, rats).

5. Detailed Plan of Procedure:

I. Human study: This phase is designed to study the bacteriology of the upper and lower respiratory tract in normals (smokers and non-smokers) and chronic bronchitics (smokers and non-smokers). The bacterial flora of expectorated sputum, oropharyngeal secretions, tracheal secretions, and secretions from the lower bronchial tree will be examined in each subject, and comparisons will be made between the different groups. Tracheal and bronchial specimens in the form of swabs and aspirates will be obtained under sterile conditions by bronchoscopy and by intubation with rubber catheters (as in bronchography). All specimens will be homogenized and plated out on appropriate media. Quantitative bacterial counts by the pour plate method will be done on the aspirates. (Please see section II on attached sheet).

II. Animal Study: Different groups of animals will be exposed to cigarette smoke and sulfur dioxide in a chamber for variable lengths of time. These times will range from acute exposure periods to periods similar to those in which changes in bronchial epithelium have been observed (C. Leuchtenberger, et al). Following these periods the animals will be exposed to a challenging dose of aerosolized bacterial (staphylococci, pneumococci) in a specially designed aerosol system.* The bacterial aerosol is generated in an air stream at one end of the apparatus, carried past the experimental animals, and then put through a filtering apparatus. This system offers a safe method for the administration of predictable doses of bacteria. At specific time intervals after exposure, groups of animals will be sacrificed; the lungs will be removed aseptically and homogenized; and bacterial counts will be done on the homogenates by

* please see section II on attached sheet

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Source: https://www.industrydocuments.ucsf.edu/docs/xhnf0000

counting colonies on agar pour plates. In this way it is possible to determine the number of bacteria cleared in a particular period of time under these special conditions. By similar experiments this past year we have constructed "time-concentration curves",** and the results show that bacteria are cleared from the lungs of normal mice and rats in an exponential manner. Ninety-five to 98 per cent of the bacteria are gone 6 hours after exposure. Time-concentration curves will be determined and compared in the animals exposed to cigarette smoke and those exposed to sulfur dioxide; these will also be compared with the control data cited above. In this way it may be possible to show the effects of these materials on the clearing of bacteria from the animals' respiratory tracts.

6. Budget Plan:

Salaries	$3,700.00
Expendable Supplies	2,193.00
Permanent Equipment	7,455.00
Overhead (15% of 1 and 2)	884.00
Other	-----
Total	$ 14,232.00

7. Anticipated Duration of Work: This study is planned for one year. The versatility of the permanent equipment, however, makes it possible in the future to apply the aerosol method to many other aspects of the problem.

8. Facilities and Staff Available: There will be adequate office and laboratory space at the medical school. There is ample area for the housing and care of laboratory animals, and culture media and basic bacteriological needs will be supplied by the hospital microbiology department. This project will require the part-time participation of a thoracic surgeon, and a bacteriology technician will also be included. It is planned that a senior medical resident will participate in the study.

9. Additional Requirements: The use of aerosolized cultures of bacteria requires maximum protection for personnel. Concerning this matter I have visited and consulted the Bacterial Warfare Center at Fort Detrick, Maryland, and the School of Public Health and Department of Industrial Hygiene at Harvard. The plan of this experiment and the protective measures we will use meet with their approval.

10. Additional Information (Including relation of work to other projects and other sources of supply): My research activities at Seton Hall will be concerned with chronic bronchitis. My major research project in the department of medicine will be the one proposed here. Along with this study I will conduct a bronchitis clinic at Seton Hall where we will continue to make observations on the natural history, living habits, and management of patients with chronic bronchitis. We hope to incorporate this information into a correlated prevalence study of bronchitis and air pollution in Hudson County, New Jersey. This will be a coordinated effort between the departments of medicine and preventive medicine. Dr. Benedict Duffy, Professor of Preventive Medicine, will head the epidemiological aspects of this investigation.

/s/ Gustave A. Laurenzi, M.D., Dir., of Project
/s/ Hugh G. Grady, Business Officer

** plots of time after exposure against the mean number of bacteria in the animals' lungs.

10035413 69

Source: https://www.industrydocuments.ucsf.edu/docs/xxxx

11. (Dr. Hockett has suggested that I include a section on past work, techniques developed, and general objectives).

The correlated study outlined here is a direct extension of the investigations which I have carried out this past year. The examination of expectorated sputum for bacteria is misleading in that secretions from the lower respiratory tract are contaminated by oropharyngeal secretions during the process of expectoration. To get a clearer picture of the bacteriology of the bronchial tree I have obtained secretions from the lower respiratory tract by bronchoscopy. These specimens show that in the normals (including many heavy cigarette smokers), the bronchial tree below the level of the carina is sterile; but in the bronchitics, oropharyngeal organisms* and pathogens are found consistently in this region. These findings suggest that any effect that cigarette smoke might have on the bronchial tree does not interfere with the normal clearing of bacteria. (This material is being prepared for a preliminary report in the New England Journal of Medicine.

In our laboratory this year we have constructed an aerosol exposure system for studying the infectivity of microorganisms suspended in air as particles. Many experiments have been done to calibrate the instrument so that with a known aerosol innoculum the number of bacteria in each animal's lungs after a fixed exposure time is predictable. We have worked with a dose by which 80,000 bacteria are deposited in each animal's lungs after thirty minutes of exposure. As noted above, timed killings after exposure have shown that 95 to 98 per cent of all the bacteria are gone in 6 hours. These studies demonstrate the remarkable capacity of the respiratory tract to clear itself of bacteria.

Chronic bronchitis is a major medical, social, and economic problem; yet our knowledge of the incidence and pathogenesis of this condition is incomplete, and methods for its control are seriously inadequate. Although the etiology of chronic bronchitis remains obscure a good deal is known about the factors which hasten a bronchitic's deterioration. By following the course of the bronchitic an important correlation has been made between the increasing disability of the bronchitic and the number of exacerbations of acute bronchitis he suffers. During exacerbations bacterial pathogens are found in the sputum in 50 to 80 per cent of the cases. From this data it appears that infection plays a major role in determining the progress of the bronchitic's deterioration: with repeated infections more and more lung tissue is damaged and pulmonary reserve is compromised. Studies are needed to show what factors upset the normal mechanisms whereby the respiratory tract is able to rid itself of bacteria. How are the bacteria able to take hold, colonize, and cause bronchial infection? Air pollution and smoking have been implicated as contributing factors. After observing bronchitics at three large medical centers the role of these factors seems more involved and unclear to me. I anticipate that the correlated human and animal study proposed here will give objective information fundamental to the entire problem. Of course this information must be tempered with the realization that these isolated effects may not be the only responsible factors.

* neisseria, non-hemolytic and green streptococci.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

191

Application For Research Grant

Date: January 22, 1958

1. Name of Investigator: M. Powell Lawton, Ph.D., and Alfred E. Goldman, Ph.D.

2. Title: A prospective and retrospective study of the psychophysiological correlates of smoking.

3. Institution & Address:

4. Project or Subject:

The motivation of smoking has received much speculation but too little scientific exploration. Our approach to this problem conceives of the smoking habit as satisfying certain physiological, psychological and social needs of the organism. The nature of some of these needs have been suggested, in part, by prior investigations. These findings, however, are based on post hoc analyses of the data and demand replication before reliability and generalization can be assumed. This study endeavors to subject the hypotheses provided by previous investigations to precise experimental test. More specifically, we propose to predict future smoking behavior of a group of high school students (over)

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Census data indicates that by age 19 years 45.3% of men have already started smoking. Since we are concerned here with prospective data it seems appropriate to limit study to male subjects in their 15th year. To reduce problems relating to sample selection it is proposed to use the entire 10th grade parameter (N = approximately 50).

A series of physiological, psychological and sociological devices will be administered during the tenth school year. Two years later each subject's smoking habits will be investigated by personal interview.

The measurement instruments given below are designed to yield data pertinent to the following spheres of inquiry.

A. Intra-personal

1. Anxiety and tension. Previous work by Matarazzo, and Lawton and Phillips, has suggested that anxiety may be a distinguishing characteristic between smokers and non-smokers. The following measures are designed to reflect gross bodily or subjectively experienced anxiety.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

4. (cont'd.) on the basis of factors suggested by prior research. Further, we seek to generate new hypotheses through retrospective analysis of the data.

5. (cont'd.)

a. Steadiness test
b. Reflex activity
c. Skin conductivity
d. Blood pressure (change in)
e. Taylor Anxiety Scale (manifest conscious anxiety)
f. Rorschach Indices: Texture, m (unconscious anxiety)
g. Rating of motoric intensity (gross motility level)
h. Adjective check list

2. Impulse expression - control. McArthur and Sommers have suggested some tendency to behavioral expression of impulses among smokers. Self-ratings and laboratory motor indices have been used with success in measuring this item.
   a. Rorschach M (cognitive inhibition)
   b. Motor inhibition task
   c. Hostility manifest expression of)
   d. Muscular pressure

3. Dependency needs. McArthur, Matarazzo, Lawton and Phillips, and Jones have all suggested that dependency needs may be important in the determination of compulsive smoking habits. Therefore, empirical measures derived from questionnaire, self-rating and Rorschach indices will be used in an attempt to differentiate the groups. In addition, data on early feeding will be sought from the mothers of the subjects as a further test of McArthur's findings regarding breast feeding.
   a. Dependency Scale; adjective checklist
   b. Rorschach Indices of dependency

4. Developmental level. All three factors mentioned above: anxiety, impulsiveness, and dependency are thought to reflect a more primitive level of psychological maturity, and suggest that a general index of developmental level may be related with the adoption of smoking. With the genetic system of scoring the Rorschach, recent research has provided a valid, reliable, and objective method of assessing psychological maturity.
   a. Genetic scoring; index of integration
   b. Genetic scoring; index of primitive thought

5. Achievement and intelligence. Matarazzo found no IQ differences between smokers and non-smokers. However, census data indicating greater incidence of smoking in lower economic groups might suggest a possible differential rate referrable to IQ. Accordingly, school IQ measurements, school grades, and various indices of socioeconomic status will be used to discriminate smokers from non-smokers.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

a. I.Q. (California Mental Maturity)
b. Grades

6. Physical strength. Height, weight and grip strength have been previously related to smoking behavior. They will be repeat for confirmation of the prior studies.
   a. Weight
   b. Grip strength

B. Interpersonal

1. Status needs. It is hypothesized that the initiation of smoking may be a compensatory phenomenon related to status needs of the individual. Therefore, the relationship of various indices of peer acceptance and leadership to eventual smoking behavior will be studied.
   a. Athletic accomplishments
   b. School extracurricular activities
   c. Sociometric rating
   d. Sociability index
   e. Hollingshead Index of Socio-economic Status

2. Attitudes. Because no prospective studies of the determinants of smoking have been done, nothing is known about the effect of specific attitudes to smoking on eventual smoking behavior. Accordingly, questionnaire items relating to smoking specifically, and to related attitudes, will be given.
   a. Parents' smoking habits
   b. Attitudes to smoking and people who smoke
   c. Attitudes to drinking, gambling, rock-and-roll, sex
   d. Asceticism - hedonism attitudes
   e. Religious attitudes

6. Budget plan:

Personnel:		1st Year		3rd Year
Professional (2)	@ $1,500.	$ 3,000.	(2)	$3,000.
Consultants		300.		300.
Technician (2, ½ time)	@ 3,000.	6,000.	(1, ½ time)	3,000.
Secretary-Clerk				500.
Expendable Supplies		200.		100.
Permanent Equipment		300.		
Overhead @ 6% of total		583.		494.
Electronic Data Processing				1,000.
Annual total		$10,383.		$8,394.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

(See attached sheet)

Salaries	_____
Expendable Supplies	_____
Permanent Equipment	_____
Overhead	_____
Other	_____
Total	_____

7. Anticipated Duration of Work:

Two work years. First year - data collection; third year - data analysis. For the second year no funds are requested.

8. Facilities and Staff Available:

M. Powell Lawton, Ph.D.
Alfred E. Goldman, Ph.D.

A resource for technicians and consultants is the University of Pennsylvania, and Temple and Jefferson Medical Colleges.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply): None

Signature /s./ M. Powell Lawton
Director of Project

Business Officer of the Institution

10035413?4

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

CROSS REFERENCE SHEET

Name or Subject: Joseph P. Lazansky

Regarding: Research Grant

SEE

Joseph F. Volker

10035413?5

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Committee:
Jacobson, Chm.
Reimann
Lynch
Kotin

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

#159R2

Activated: 9/1/57
Renewed: 9/1/58*
(Cf. #74
Activated: 6/1/55
Renewed: 6/1/56

* (Anniversary date changed to 1/1)

Renewal
Application For Research Grant

Date: Nov. 27, 1959

1. Name of Investigator: Cecilie Leuchtenberger, Ph.D.

2. Title: Senior Biologist and Cytochemist

3. Institution & Address: Children's Cancer Research Foundation
35 Binney Street
Boston, Mass.

4. Project or Subject: A correlated histological, cytological and cytochemical study of the tracheobronchial tree from mice exposed to cigarette smoke.

The interrelation between host factors and exposure to cigarette smoke in the development of bronchial lesions in mice.

5. Detailed Plan of Procedure:

A. SPECIFIC AIMS

The purpose of this project is the continuation and extension of the study of the sequence of events occurring in the tracheobronchial tree of mice exposed to cigarette smoke. Correlated histological, cytological and cytochemical investigations will be carried out and combined with the application of newer quantitative cytochemical techniques, such as microspectrophotometry and interference microscopy. It is felt that these methods, particularly when used simultaneously with other methods, such as fluorescence microscopy and tissue culture, will be of great help when attempting to explore the interrelationship between host factors and exposure to cigarette smoke in the development of bronchial lesions in mice.

Results of previous studies done in our laboratory on the project, which are concerned with the histopathological, cytopathological and cytochemical changes in the tracheobronchial tree and lungs of mice after exposure to cigarette smoke, have been reported at National and International Congresses and have been published under the titles:

I. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE.
Bronchitis with Atypical Epithelial Changes in Mice Exposed to Cigarette Smoke
Cancer, Vol. 11, No. 3, May-June, 1958

II. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE SEQUENCE OF EVENTS IN THE BRONCHIAL EPITHELIUM FROM MICE EXPOSED TO CIGARETTE SMOKE.
Acta Union Internationale contre le Cancer, Vol. XV, Nos. 3-4, 1959

1003541376

Source: https://www.industrydocuments.ucsf.edu/docs/tfvh0000

III. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE.
Varying Responses of Major Bronchi to Cigarette Smoke, Absence of Bronchogenic Carcinoma after Prolonged Exposure, and Disappearance of Bronchial Lesions after Cessation of Exposure.
Cancer (In press.)

IV. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE.
Unaltered Incidence of Grossly Visible Adenomatous Lung Tumors in Female CF_1 Mice after Prolonged Exposure to Cigarette Smoke.
Cancer (In press.)

One of the main problems which seems to us to be of particular importance concerns the interrelation between host factors and exposure to cigarette smoke in the development of the bronchial lesions in mice. A thorough investigation of the possible role of the host factors is revealed by the findings of our previous studies as being both warranted and particularly pertinent. The data which are reported in the paper Varying Responses of Major Bronchi to Cigarette Smoke, Absence of Bronchogenic Carcinoma after Prolonged Exposure, and Disappearance of Bronchial Lesions after Cessation of Exposure disclosed that the variability in response of the major bronchi from mice after exposure to cigarette smoke is one of the most striking results of our studies. Differences in response were observed regardless of whether groups of mice were exposed to a few cigarettes for a short period or to many hundreds of cigarettes for nearly a lifespan. Since, in each group, all mice were handled in a standardized manner, host susceptibility must be taken into consideration when an attempt is made to interpret the relationship between changes in the major bronchi and exposure to cigarette smoke. While a differential response might be expected from mouse to mouse in a group of mice after exposure to smoke from a few cigarettes, it is indeed puzzling that such a wide spectrum of response, including resistance, persisted on chronic exposure. In some of the mice, even after exposure to over 1,000 cigarettes for nearly their whole lifespan, the bronchi were indistinguishable from those of nonexposed controls. In this connection it should be mentioned that the frequency of resistant cases increased considerably in the latter part of 1957 as compared with that encountered during the first phase of our study (1956). In 1958 we even observed a group of 45 mice without any lesions in the major bronchi, despite the fact that all 45 mice were exposed daily to smoke from three cigarettes for over three months. The increase in resistance was equally observed among groups of mice exposed to cigarette smoke in the smaller as well as in the 30% larger smoking chamber. In view of the fact that, to the best of our knowledge, all experimental conditions were kept the same in our laboratories during the years 1956-1958, we are unable to explain the change in incidence. However, since the mice were kept on a commercial diet which was repeatedly replenished (utilizing the same brand), changes in the composition of the food, including the addition of antibiotics and preservatives, cannot be excluded. One may also perhaps suggest that changes were made in the preparation of the cigarettes during the later periods of our experiments which may be responsible for the difference in the results. Whatever may develop as the cause for the increase of refractory cases, there can be no doubt that the differences in response, including complete resistance of the major bronchi to cigarette smoke, were encountered at all periods

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of our study. For example, among 23 cases presented in our first paper, six mice were found to have no alterations after exposure to cigarette smoke. The wide spectrum of findings and the independence of dose and duration of exposure to cigarette smoke would seem to detract from the importance of cigarette smoke as an injurious agent invariably affecting the major bronchi and would, rather, point to contributing factors characteristic for the individual animal. Actually, the difference in response of mice to cigarette smoke, including the considerable number of completely refractory cases, is not so surprising, but is in accordance with experience obtained in humans. Examination of the tracheobronchial tree of heavy smokers, as carried out by Auerbach and his associates, demonstrated a wide range of alterations, including cases without lesions. Furthermore, statistical data on the relationship between bronchogenic carcinoma and cigarette smoking have shown that, even among heavy smokers, only a relatively small percentage develop bronchogenic carcinoma.

Thus it appears that, in cases in which a harmful effect on the tracheobronchial tree has been observed after cigarette smoke, the cigarette smoke may not be solely responsible, but that there may be other elements within the host which may render the tissue susceptible to smoke. The concept that the tracheobronchial tree may, indeed, need a "special conditioning" for the injurious action of the tobacco smoke is supported by our data on mice - that the difference in response and resistance of the bronchi to cigarette smoke is independent of dose and length of exposure.

While it is impossible, at present, to relate any particular factor/or factors of the host to the resistance or susceptibility of the tracheobronchial tree to cigarette smoke, the investigation of agents possibly carried by the host, which may contribute to the injury of the bronchi, would seem of great interest. Among the many possible host factors, viruses deserve special consideration - the more so since they occur with a certain frequency in mice and are capable of producing respiratory lesions.

It is well known that mice may be carriers of latent viruses which, under certain circumstances, may provoke alterations. In view of the increasing recognition of the dual role of viruses as either cytolytic agents or stimulators of cell proliferation, which may even lead to tumor formation, the investigation of viruses in combination with exposure to cigarette smoke seems to us particularly worthwhile. There are two main lines which we are investigating at present. The first is concerned with the detection of latent viruses in mice prior to exposure to cigarette smoke. It will be of interest to learn whether mice carrying a latent virus will exhibit a different response in the bronchi from that of mice free from virus. The second line is concerned with the effect of exposure to virus in addition to the exposure to cigarette smoke. If our concept is correct that viruses may act as co-factors in the production of bronchial lesions, then mice which carry latent viruses prior to exposure to smoke, or which are infected with viruses, should show frequent and perhaps more severe lesions, while mice free of virus should either be refractory or should show mild lesions.

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B. Material and Methods: Essentially the same methods will be employed as in the previous studies. However, in addition to the microscopic, microspectrophotometric methods, special methods concerned with the detection and identification of viruses in mice after exposure to cigarette smoke, and after infection with respiratory viruses will be utilized, such as tissue culture and fluorescence microscopy. Attention will also be given to the possible role of nutritional factors.

Source: https://www.industrydocuments.ucsf.edu/docs/jfvm0000

6. Budget Plan:

Salaries	$30,340.00
Expendable Supplies	1,300.00
Permanent Equipment	-
Overhead (15%)	4,821.00
Other (Travel)	500.00
Total	$36,961.00

7. Anticipated Duration of Work:

Three to five years

8. Facilities and Staff Available:

The facilities which are available for these studies is an established laboratory of cytochemistry (Head, Dr. Cecilie Leuchtenberger) and suitable animal quarters in the Children's Cancer Research Foundation. The cytochemical laboratories are well-equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are: (1) four microspectrophotometric apparatuses*; (2) one ultraviolet microspectrophotometer; (3) six research microscopes; (4) two phase microscopes; (5) two photomicrographic set ups; (6) one Beckman spectrophotometer; (7) one freeze drying apparatus for the fixation and embedding of tissues; (8) two interference microscopes; (9) tobacco research laboratory including newly designed and built smoking machine. There is also a team of workers (professional and technical) comprising cytochemist, pathologists, and research assistants.

* also fluorescence microscope.

9. Additional Requirements:

Personnel

1.	Pathologist	$13,000 + 3% Soc. Sec.	$13,144
2.	Experienced cytologist at Ph.D. level	6,000 + 3% " "	6,144
3.	(2) research technicians trained in histological procedures and animal experimentation	7,200 + 3% " "	7,344
4.	Part time secretary and research librarian	1,200 + 3% " "	1,236
5.	Animal caretaker and glass washer	2,400 + 3% " "	2,472
Total Salaries and Social Security			$30,340

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

9. Additional Requirements (cont'd.):

Expendable Supplies

1. Animals and animal food (approximate estimation)	$500
2. Cigarettes and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)	800
Total expendable supplies	$1,300

10. Additional Information (Including relation of work to other projects and other sources of supply): - - -

/s./ Cecilie Leuchtenberger
Director of Project -
(Associate Professor of Cytology and Biology)
Senior Biologist and Cytochemist

/s./ Sidney Farber
President

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE #159R1
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y. (See #159 activated 9/1/57)
Also #74 - Activated 6/1/55
Renewed 6/1/56

Application For Research Grant **RENEWAL to begin Sept. 1, 1958**

Date: April 9, 1958

1. Name of Investigator: Cecilie Leuchtenberger, Ph. D.

2. Title: Associate Professor of Cytology and Biology

3. Institution & Address:
Institute of Pathology
Western Reserve University
2085 Adelbert Road
Cleveland 6, Ohio

4. Project or Subject: A correlated histological, cytological, and cytochemical study of the tracheo-bronchial tree from mice exposed to cigarette smoke.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Specific Aims

The purpose of this project is the continuation and extension of the histological, cytological, and cytochemical studies of the tracheobronchial tree from mice exposed to cigarette smoke. Previous studies done in our laboratory on the same problem have revealed distinct histological, cytological, and cytochemical changes in the tracheobronchial tree from mice exposed to cigarette smoke. The experimental conditions and the results obtained so far have been described in progress reports sent to the Tobacco Industry Research Committee (See especially the Progress Report of June 1956 to March 1957). Furthermore, a paper entitled: "Quantitative Determination of Nucleoproteins by Microspectrophotometry and of Dry Mass by Interference Microscopy in Bronchial and Lung Cells from Mice Exposed to Cigarette Smoke" has been presented by Dr. Cecilie Leuchtenberger, Dr. Rudolf Leuchtenberger, and Mr. Paul F. Doolin at the American Association for Cancer Research in Chicago on April 12, 1957. In addition, the following papers have been presented; and will be presented in the near future:

1. "Cytological and Cytochemical Changes in the Epithelial Cells of the Tracheobronchial Trees from Mice Exposed to Cigarette Smoke" by Dr. Cecilie Leuchtenberger, Dr. Rudolf Leuchtenberger, and P. F. Doolin at the IXth International Congress for Cell Biology in St. Andrews/Scotland, Aug. 28 - Sept. 3, 1957.
2. "Changes in the Bronchial Epithelium of Mice after Exposure to Cigarette Smoke. A correlated Histological, Cytological, and Cytochemical Study." by

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(continued from page 1)

Dr. Cecilie Leuchtenberger, Rudolf Leuchtenberger, M.D., Dr. William Zebrun, and Patricia Shaffer; at the Meeting of the American Association of Pathologists and Bacteriologists in Cleveland on April 24 and 25, 1958.

3. (see also 3a) "A Correlated Histological, Cytological, and Cytochemical Study of the Sequence of Events in the Bronchial Epithelium from Mice Exposed to Cigarette Smoke" by Dr. Cecilie Leuchtenberger, Dr. Rudolf Leuchtenberger, and Dr. William Zebrun; at the VIIIth International Cancer Congress in London, July 6-12, 1958.

There are several main problems which warrant further thorough investigation:

(1) A follow-up of the early cytochemical and histopathological changes, especially of the atypical hyperplasia, the squamous cell metaplasia and "carcinoma in situ." It seems of importance to find out whether these alterations of the epithelium are reversible, stationary or may lead to invasive cancer. Consequently mice will be exposed for varying periods to cigarette smoke and the sequential changes in the tracheobronchial tree will be studied during the whole life span of the mice. Such experiments may also yield information of a more general nature as to the significance of "carcinoma in situ" for the evolution of cancer.

(2) Since inflammatory processes such as bronchitis and peribronchitis were observed very frequently in the mice exposed to cigarette smoke, the question arises whether the atypical epithelial changes may not just be an expression of these bronchitic alterations. In order to obtain an answer to this question, it would be desirable to induce bronchitis and peribronchitis in mice by means other than cigarette smoke.

(3) In order to test the theory proposed by some scientists that cigarette smoke acts as a carcinogen, it would be of significance to expose mice to a known carcinogen, such as benzpyrene, and then study the sequential histological, cytological, and cytochemical changes exactly as it is done after exposure to cigarette smoke.

5. B. Material and Methods

The material and methods will be essentially the same as in the previous studies. However, in order to permit a larger scope of the studies, greater number of mice will be utilized, and a second smoking machine has been built.

3a. "A Correlated Histological, Cytological, and Cytochemical Study of the Tracheobronchial Tree and Lungs from Mice Exposed to Cigarette Smoke.
I. Bronchitis with Atypical Epithelial Changes in Mice Exposed to Cigarette Smoke." by Dr. Cecilie Leuchtenberger, Dr. Rudolf Leuchtenberger, and P. F. Doolin. Will appear in the May-June issue of the Journal Cancer, 1958.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	$17137.50
Expendable Supplies	1300.00
Permanent Equipment	----
Overhead	2765.62
Other (Travel)	500.00
Total	$21703.12

7. Anticipated Duration of Work: **Three to five years**

8. Facilities and Staff Available:

The facilities which are available for these studies is an established laboratory of cytochemistry (Head, Dr. Cecilie Leuchtenberger) and suitable animal quarters in the Institute of Pathology, Western Reserve University. The cytochemical laboratories are well-equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are: (1) Four microspectrophotometric apparatuses; (2) one ultraviolet microspectrophotometer; (3) six research microscopes; (4) two phase microscopes; (5) two photomicrographic setups; (6) one Beckman spectrophotometer; (7) one freeze-drying apparatus for the fixation and embedding of tissues; (cont'd reverse side)

9. Additional Requirements: **See reverse side**

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature
Director of Project /s/ Cecilie Leuchtenberger, Ph. D.
Associate Professor of Cytology and Biology

Business Officer of the Institution /s/ [illegible] Burke, Asst. Comptroller

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Continued No. 8

(8) two interference microscopes; (9) tobacco research laboratory including newly designed and built smoking machine.

There is also a team of workers (professional and technical) comprising cytochemist, pathologists and research assistants.

No. 9 - Additional Requirements

Personnel

1.	Experienced cytologist at Ph. D. level	$ 6000	2½	Soc. Sec.	$6094.50
2.	(2) Research technicians trained in histological procedures and animal experimentation	7200	2½	" "	7362.00
3.	Part-time secretary and research librarian	1200	2½	" "	1227.00
4.	Animal caretaker and glassware washer	2400	2½	" "	2454.00
		$16800			$17137.50

Expendable supplies

1.	Animals and animal food (approximate estimation)	$ 500.00
2.	Cigarettes and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)	800.00
		$ 1300.00

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

74

4 Budget Plan
for first year

Application For Research Grant

Overhead
Other

Date: March 11, 1955

1. Name of Investigator: Cedilie Leuchtenberger, Ph.D.

2. Title: Associate Professor of Cytology, School of Medicine
Western Reserve University

3. Institution & Address: Institute of Pathology
Western Reserve University
Cleveland 6, Ohio
2085 Adelbert Road

4. Project or Subject:
Quantitative Analysis of Nucleoproteins in Tissues from Animals Subjected to Tobacco Smoke by Microspectrophotometry and Interference Microscopy Correlated with Cytological and Histological Studies.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Specific Aim. The purpose of this project is a simultaneous study of the cytochemical, cytological and histological alterations which may be induced in organs of animals which have been exposed to tobacco smoke.

Since the nucleoproteins constitute essential building stones of all cells in every tissue and are closely associated with their genetic properties, growth and metabolism, it is felt that an investigation of desoxyribose nucleic acid (DNA), ribose nucleic acid (RNA) and proteins in cells under the influence of tobacco smoke may reveal changes prior to their morphological manifestations.

The recent development of microspectrophotometric methods and interference microscopy which allow the quantitative estimation of nucleoproteins and dry mass determination in single cells in situ directly under the microscope make it possible to approach a study at such a sensitive level. Furthermore, previous studies by these methods on various normal and malignant tissues in both animals and humans (see enclosed reprints) have revealed that microspectrophotometry is indeed an excellent method for detecting submorphological changes.

Closely correlated with these quantitative cytochemical analyses will be detailed cytological and histological examinations of all the tissues concerned. In the cytological studies, carefub attention will be given to the possible occurrence of mitosis in otherwise resting tissues and also to any abnormalities which may occur in such cellular elements as nucleoli, nuclei and cytoplasm. In the histological studies, special emphasis will be given to possible hyperplastic and degenerative changes in the cardiovascular system, the respiratory system, the liver, kidneys, adrenals and reproductive organs.

(continued on next page)

/s/ C. L.
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/fbm0000

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#5 continued.

B. Materials and Methods. Mice of known genetic strains of both sexes at various ages will be exposed to tobacco smoke in a smoking machine similar to that of Essenberg (Science 116:561,1952) for varying intervals. Control animals of the same strain, sex and age will be put into a control chamber for the same lengths of time.

Both control and experimental animals will be kept on a stock diet, a diet rich in protein and a diet rich in fat and also subjected to various stresses (such as work on a treadmill).

Each group and controls will be sacrificed at varying intervals and tissues will be removed for cytochemical, cytological and histological examination.

For the quantitative estimation of the nucleoproteins, visible and ultraviolet microspectrophotometry will be employed. The validity of these microspectrophotometric methods has been established by comparing them with other standard methods (see enclosed reprints entitled "The Nucleoproteins in Cell Growth and Division" and "Critical Evaluation of Feulgen Microspectrophotometry for Estimating Amount of DNA in Cell Nuclei".)

For the determination of the mass of cells and cell parts, the interference microscopic method will be employed according to the technic of Davis and Wilkins (Nature 169:541,1952).

#9 ~~continued.~~ Additional Requirements

PERSONNEL

1.	Experienced Cytologist at Ph.D. level	$6000/yr. + 2% Soc. Sec. =	$6084
2.	Research technician trained in histological procedures and animal experimentation	3600 " " " "	3672
3.	Part time secretary and research librarian	1200 " " " "	1224
4.	Animal caretaker and glassware washer	2400 " " " "	2448
		Total	$13428

PERMANENT EQUIPMENT

1.	Interference microscope	(Approximate estimation)	$ 3000
2.	Smoking apparatus, treadmill, mazes	"	1500
3.	Animal cages	"	400
4.	Research microscope	"	1500
			$ 6400

EXPENDABLE SUPPLIES

1.	Animals and animal food	(Approximate estimation)	$ 500
2.	Special quartz slides, and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)	"	800
			$ 1300

REPRINTS ENCLOSED

1. The Nucleoproteins in Cell Growth and Division, Cecilie Leuchtenberger, in Statistics and Mathematics in Biology, Ed. by Oscar Kempthorne, Theodore A. Bancroft, John W. Gowen, and Jay L. Lush. Iowa State College Press. Ames. 1954.

2. A Microspectrophotometric Study of the Desoxyribose Nucleic Acid Content in Cells of Normal and Malignant Human Tissues, C. Leuchtenberger, R. Leuchtenberger and A. M. Davis. Am. J. Path. 30, 65-85 (1954).

3. Critical Evaluation of Feulgen Microspectrophotometry for Estimating Amount of DNA in Cell Nuclei. C. Leuchtenberger. Science, 120, 1022-1023 (1954).

4. The Desoxypibosenucleic Acid Content in Spermatozoa of Fertile and Infertile Human Males. C. Leuchtenberger, F. Schrader, D. R. Weir and Doreen P. Gentile. Chromosoma 6, 61-78 (1953).

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

for first year

Salaries	$13,428
Expendable Supplies	1,300
Permanent Equipment	6,400
Overhead	1,771124
Other	300
Total	$23,199.24

7. Anticipated Duration of Work:

Three years

8. Facilities and Staff Available: The facilities which are available for these studies is an established laboratory of cytochemistry (Head, Dr. C. Leuchtenberger) and suitable animal quarters in the Institute of Pathology at Western Reserve University. The cytochemical laboratories are well equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are (1) Four microspectrophotometric apparatuses; (2) one ultraviolet microspectrophotometer; (3) five research microscopes; (4) one phase microscope; (5) one photomicrographic setup; (6) one Beckman spectophotometer; (7) one freeze-drying apparatus for the fixation and embedding of tissues.

There is also a team of workers (professional and technical) comprising cytochemists, pathologists and research assistants.

9. Additional Requirements:

See preceding page.

5. (Attach list of Procedures (Use reverse side if additional space is needed)

10. Additional Information (Including relation of work to other projects and other sources of supply):

The purpose of this project is a simultaneous study of the cytochemical, cytological and histological alterations which may be induced in organs or animals which have been exposed to tobacco smoke.

Since the nucleoproteins constitute essential building stones of all cells in every tissue and are closely associated with their genetic properties, growth and activities, it is felt that an investigation of desoxyribose nucleic acid (DNA), ribose nucleic acid (RNA) and proteins in cells under the influence of tobacco smoke may reveal changes prior to their morphological manifestations.

The recent development of microspectrophotometric methods and interference microscopy which allow the quantitative estimation of nucleoproteins and dry mass concentration in single cells in situ directly under the microscope make it possible to approach a study at such a sensitive level. Furthermore, previous studies by these methods on various normal and malignant tissues in both animals and humans (see enclosed reprints) have revealed that microspectrophotometry is indeed an excellent method for revealing submorphological changes.

Closely correlated with these quantitative cytochemical analyses will be detailed cytological and histological examinations of all the tissues concerned. In the cytological studies, careful attention will be given to the possible occurrence of mitosis in stimulus resting tissues and also to any abnormalities which may occur in such cellular elements as nucleoli, nuclei and cytoplasm. In the histological studies, special emphasis will be given to the changes in the cardiovascular system, the respiratory, adrenals and reproductive organs.

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Signature /s/ Ceelie Leuchtenberger, Ph.D.
Director of Project

/s/ S. F. Agnew
Business Officer of the Institution

S. F. Agnew, Cc [illegible]

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Source: https://www.industrydocuments.ucsf.edu/docs/fvm0000

CONFIDENTIAL

TIRC Grant #74

Progress Report #1

Dr. Cecilie Leuchtenberger
Western Reserve University

November 30, 1955

"Quantitative Analysis of Nucleoproteins in Tissues from Animals Subjected to Tobacco Smoke by Microspectrophotometry and Interference Microscopy Correlated with Cytological and Histological Studies"

The activities of the first six months were concerned mainly with planning our experiments and setting up a laboratory to carry out these experiments. Furthermore a number of pilot experiments have been carried out in which mice were exposed to smoke for varying periods of time. In addition, our efforts have been directed toward establishing a normal baseline to compare with possible eventual changes in mice exposed to tobacco smoke. Extensive histological, cytological and cytochemical studies have been started on normal tissues from the respiratory system. Similar studies have also been started on the same tissues from tobacco exposed mice. It is obviously too early to give any results at the present time.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application for Research Grant

-RENEWAL - 74R1

Date: April 2, 1956

1. Name of Investigator:
Cecilie Leuchtenberger, Ph.D.

2. Title:
Associate Professor of Cytology and Biology

3. Institution & Address:
Institute of Pathology
Western Reserve University
Cleveland 6, Ohio
2085 Adelbert Rd.

4. Project or Subject:
Quantitative Analysis of Nucleoproteins in Tissues from Animals Subjected to Tobacco Smoke by Microspectrophotometry and Interference Microscopy Correlated with Cytological and Histological Studies.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Specific Aim. The purpose of this project is a simultaneous study of the cytochemical, cytological and histological alterations which may be induced in organs of animals which have been exposed to tobacco smoke.

Since the nucleoproteins constitute essential building stones of all cells in every tissue and are closely associated with their genetic properties, growth and metabolism, it is felt that an investigation of desoxyribose nucleic acid (DNA), ribose nucleic acid (RNA) and proteins in cells under the influence of tobacco smoke may reveal changes prior to their morphological manifestations.

The recent development of microspectrophotometric methods and interference microscopy which allow the quantitative estimation of nucleoproteins and dry mass determinations in single cells in situ directly under the microscope make it possible to approach a study at such a sensitive level. Furthermore, previous studies by these methods on various normal and malignant tissues in both animals and humans have revealed that microspectrophotometry is indeed an excellent method for detecting submorphological changes.

Closely correlated with these quantitative cytochemical analyses will be detailed cytological and histological examinations of all the tissues concerned. In the histological studies, special emphasis will be given to possible

(Cecilie Leuchtenberger, Ph.D.)

/s/ E. P. Agney by E. M. Burke
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/jfvn0000

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hyperplastic and degenerative changes in the cardiovascular system, the respiratory system, the liver, kidneys, adrenals and reproductive organs.

B. Materials and Methods. Mice of known genetic strains of both sexes at various ages will be exposed to tobacco smoke in a smoking machine similar to that of Essenberg (Science 116:561,1952) for varying intervals. Control animals of the same strain, sex and age will be put into a control chamber for the same lengths of time.

Both control and experimental animals will be kept on a stock diet, a diet rich in protein and a diet rich in fat and also subjected to various stresses (such as work on a treadmill).

Each group and controls will be sacrificed at varying intervals and tissues will be removed for cytochemical, cytological and histological examination.

For the quantitative estimation of the nucleoproteins, visible and ultra-violet microspectrophotometry will be employed. The validity of these microspectrophotometric methods has been established by comparing them with other standard methods.

For the determination of the mass of cells and cell parts, the interference microscope method will be employed according to the technic of Davis and Wilkins (Nature 169:541,1952).

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO [illegible]
350 FIFTH [illegible]

6. Budget Plan:

	Salaries	$13,428.00
Applica	Expendable Supplies	1,300.00
	Permanent Equipment	
	Overhead	2,254.20
	Other	300.00*
	Total	$17,282.20

*Travel-Attendance at Scientific Meetings

7. Anticipated Duration of Work: [illegible]

Two Years

8. Facilities and Staff Available: [illegible] Cytology and Biology

The facilities which are available for these studies is an established laboratory of cytochemistry(Head,Dr.C.Leuchtenberger) and suitable animal quarters in the Institute of Pathology, Western Reserve University. The cytochemical laboratories are well equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are (1) Four microspectrophotometric apparatuses; (2) one mm ultraviolet microspectrophotometer; (3) five research microscopes; (4) one phase microscope; (5) one photomicrographic setup;(6) one Beckmann spectrophotometer;(7)one freeze-drying apparatus for the fixation and embedding of tissues(8)one interference microscope;(9)tobacco research laboratory including newly designed smoking machine. There is also a team of workers (professional and technical) comprising cytochemists, pathologists and research assistants.

9. Additional Requirements: [illegible]

See reverse side

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

10. Additional Information (Including relation of work to other projects and other sources of supply):

[illegible] The purpose of this project is a simultaneous study of the cytological, cytological and biochemical alterations which may be induced in tissues of animals which have been exposed to tobacco smoke.

[illegible] Since the carcinogenic constituents of tobacco smoke will result in early tissue and cell changes, and since the development of cancer, growth and metastasis, it is felt that an investigation of the nucleic acid (DNA), ribose nucleic acid (RNA) and proteins in cells will influence of tobacco smoke by several routes prior to their morphological manifestations.

The recent development of microspectrophotometric methods [illegible] investigation of cells allow the quantitative determination of nucleoproteins and [illegible] constituents in single cells in situ directly under the microscope. It should be possible to estimate a small [illegible] such a sensitive level. Furthermore, [illegible] previous studies on various normal and malignant tissues in [illegible] and human have revealed that microspectrophotometry is [illegible] and [illegible] cytological changes.

[illegible] permanent and [illegible] quantitative cytochemical [illegible] will be studied cytological and histological examinations of all the tissues [illegible]. In the histological studies, special [illegible] will be given to [illegible]

Signature /s/ Cecilie Leuchtenberger, Ph.D
Director of Project

/s./ S. F. Agnew by H. N. Burke
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

No. 9 - Additional Requirements

PERSONNEL

1. Experienced Cytologist at Ph.D. level	$6000/yr. plus 2% Soc.Sec.	=	$ 6084
2. Research technician trained in histological procedures and animal experimentation	3600 " " " " "	=	3672
3. Part time secretary and research librarian	1200 " " " " "	=	1224
4. Animal caretaker and glassware washer	2400 " " " " "	=	2448
			$13428

EXPENDABLE SUPPLIES

1. Animals and animal food	(Approximated Estimation)	=	500
2. Special quartz slides, and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)			800
			$ 1300

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #74

Progress Report #2

Dr. Cecilie Leuchtenberger
Western Reserve University

April 1, 1956

"Quantitative Analysis of Nucleoproteins in Tissues from Animals Subjected to Tobacco Smoke by Microspectrophotometry and Interference Microscopy Correlated with Cytological and Histological Studies"

From the time the grant was received until now (a period of 10 months), a special laboratory has been set up to carry out the studies as outlined in topic No. 5 of the application form.

Preliminary trials with the Essenberg smoking machine were not too satisfactory because the performance of the machine was erratic and could not be standardized. Therefore it was necessary to redesign this machine and improve it. A photograph of the modified machine is enclosed with a short description. We feel that this newly built machine which has been in operation for a few months is satisfactory because mice can be exposed to cigarette smoke under standardized and reproducible conditions.

Up to now, approximately 50 mice have been studied after exposure to cigarette smoke for period varying from 1 to 60 days at varying intervals during each day and with varying numbers of cigarettes. The same number of mice were kept in the control chamber (see "L" in photograph). For each experiment, animals from both the experimental and control groups were killed at the same time and appropriate tissues were immediately removed for fixation.

Histological, cytological and microspectrophotometric studies have been made on the bronchial and lung tissues from these animals. Up to the present time, no significant microscopic differences have been found in these tissues between the groups exposed to cigarette smoke and the ones not exposed.

Extensive microspectrophotometric determinations of the desoxyribose nucleic acid (DNA) content have been made in more than 2000 cells from the bronchi and lungs of the smoked and non-smoked animals. Again, no significant differences have been found between the exposed and non-exposed animals. Both these groups show surprisingly constant diploid amounts of DNA in the cells from the bronchi and lungs. Although it is obviously too early to draw any conclusions on the basis of these data, it seems justified to say that up to the present, no hyperplasia has been observed in mice exposed to cigarette smoke under the conditions described above.

These DNA studies will be continued because it is felt that longer periods of exposure may perhaps result in some changes. An excellent baseline has now been established for comparison with the future long term experiments planned. To the best of our knowledge, no such data have been reported.

Similar studies have been started for dry mass and protein determinations. However, due to the great difficulty in elaborating proper technics for these special tissues, the data are still too scanty to permit even a preliminary analysis now.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Description of the Smoking Machine used for the Experiments Described in the enclosed Progress Report.

This machine is made of plastic and was built at the Institute of Pathology, Western Reserve University. The disc which transports the cigarettes was built by Dr. J. Essenberg at the University of Chicago.

By means of the vacuum pump (A), air is withdrawn from the smoking chamber (E) at outlet (B) and control chamber (L) at outlet (C). These two chambers are separated by an airtight plastic partition (D).

Air passes from the outside through a Seitz filter (F) into the cigarette house (G), through a cigarette (H), into the smoking chamber (E) by means of an opening at (I) in front of a burning cigarette (H).

An electric clock (J) automatically advances at selected intervals the disc (K) that transports the cigarettes on the disc into burning position. Also an electric fuse (H') tripped by a micro-switch, ignites a cigarette when it comes into burning position.

Air is drawn into the control chamber (L) through a Seitz filter (M).

Flowmeters are located at (N) and (O). These measure the rate of flow of gas through the smoking chamber and control chamber respectively. Thus an estimate of air volume passing through the chambers is possible. At (P) and (Q) are the openings used for determining air pressures in both cages by a manometer. These openings are also used for collection of oxygen and carbon dioxide samples.

Experimental mice are placed in wire baskets on the rack (R). Control animals are placed in wire baskets on the rack (S).

A cotton filter is located at (B) and also at (T). These remove tars from the gas before they are released into the room. A room exhaust fan is located next to the pump (A).

A rubber gasket is located between the cigarette house (G) and smoking chamber (E). Also, a rubber gasket is located between the lid (with handles) and the two chambers of the apparatus (experimental (E) and control (L)).

Screws, like (U) and (V) provide an airtight seal.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y. #159

~~XXXXX~~
Application For Research Grant (Refer to #74, activated June 1, 1955 and renewed June 1, 1956)

Date: April 22, 1957

1. Name of Investigator: Cecilie Leuchtenberger, Ph.D.

2. Title: Associate Professor of Cytology and Biology

3. Institution & Address: Institute of Pathology
Western Reserve University
2085 Adelbert Road
Cleveland 6, Ohio

4. Project or Subject: A correlated histological, cytological and cytochemical study of the tracheo-bronchial tree from mice exposed to cigarette smoke.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Specific Aims

The purpose of this project is the continuation and extension of the histological, cytological and cytochemical studies of the tracheo-bronchial tree from mice exposed to cigarette smoke. Previous studies done in our laboratory on the same problem have revealed distinct histological, cytological and cytochemical changes in the tracheo-bronchial tree from mice exposed to cigarette smoke. The experimental conditions and the results obtained so far have been described in progress reports sent to the Tobacco Iidustry Research Committee (see especially the Progress Report of June 1956 to March 1957). Furthermore, a paper entitled: "Quantitative Determination of Nucleoproteins by Microspectrophotometry and of Dry Mass by Interference Microscopy in Bronchial and Lung Cells from Mice Exposed to Cigarette Smoke" has been presented by Dr. Cecilie Leuchtenberger, Dr. Rudolf Leuchtenberger and Mr. Paul F. Doolin at the American Association for Cancer Research in Chicago on April 12, 1957.

There are several main problems which warrant further thorough investigation:

(1) A follow-up of the early cytochemical and histopathological changes, especially of the atypical hyperplasia, the squamous cell metaplasia and "carcinoma in situ". It seems of importance to find out whether these alterations of the epithelium are reversable, stationary or may lead to invasive

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

No. 5 - Continued

cancer. Consequently, mice will be exposed for varying periods to cigarette smoke and the sequential changes in the tracheo-bronchial tree will be studied during the whole life span of the mice. Such experiments may also yield information of a more general nature as to the significance of "carcinoma in situ" for the evolution of cancer.

(2) Since inflammatory processes such as bronchitis and peribronchitis were observed very frequently in the mice exposed to cigarette smoke, the question arises whether the atypical epithelial changes may not just be an expression of these bronchitic alterations. In order to obtain an answer to this question, it would be desirable to induce bronchitis and peribronchitis in mice by means other than cigarette smoke.

(3) In order to test the theory proposed by some scientists that cigarette smoke acts as a carcinogen, it would be of significance to expose mice to a known carcinogen, such as benzpyrene, and then study the sequential histological, cytological and cytochemical changes exactly as it is done after exposure to cigarette smoke.

5. B. Material and Methods

The material and methods will be essentially the same as in the previous studies. However, in order to permit a larger scope of the studies, greater number of mice will be utilized and a second smoking machine will be built and used. Following a suggestion of Dr. Paul Kotin of the University of Southern California, the second smoking machine will be modified in such a way that the mice can be exposed to intermittant cigarette smoke. For the cytochemical analysis microspectrophotometry and interference microscopy will be utilized and it is also hoped that correlating with these methods some electron-microscopic studies can be done.

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Source: https://www.industrydocuments.ucsf.edu/docs/tvmd0000

6. Budget Plan:

Salaries		17137.50
Expendable Supplies		1300.00
Permanent Equipment	#1	1000.00
Overhead	(15%)	2990.62
Other	#2	500.00
Total		$ 22928.12

#1 Specially designed smoking machine.

#2 Travel (attendance at scientific meetings; consultations.)

7. Anticipated Duration of Work: Three to five years.

8. Facilities and Staff Available:

The facilities which are available for these studies is an established laboratory of cytochemistry (Head, Dr. C. Leuchtenberger) and suitable animal quarters in the Institute of Pathology, Western Reserve University. The cytochemical laboratories are well-equipped for all tissue work, as well as for the analysis of intracellular substances. Some of the major items of permanent equipment are: (1) four microspectrophotometric apparatuses; (2) & one ultraviolet microspectrophotometer; (3) six research microscopes; (4) two phase microscopes; (5) two photomicrographic setups; (6) one Beckman spectrophotometer; (7) one freeze-drying apparatus for the fixation and embedding of tissues; (cont'd next page)

9. Additional Requirements:

see next page

10. Additional Information (Including relation of work to other projects and other sources of supply):

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Signature /s/ Cecilia Leuchtenberger, Ph.D.
Director of Project Associate Professor of Cytology and Biology

/s/ S. F. Agnew, Comptroller
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

(8) two interference microscopes; and (9) tobacco research laboratory incldding newly designed smoking machine. However, the animal quarters will have to be extended to provide sufficient facilities for expanding the number of experiments now deemed necessary to pursue these types of investigations. Furthermore, we have one especially designed smoking machine, but again, to expand the investigations, at least one more machine is needed. There is also a team of workers (professional and technical) comprising cytochemist, pathologists and research assistants.

No. 9 - Additional Requirements

Personnel

1.	Experienced cytologist at Ph.D. level	$6000	+ 2½ Soc. Sec.	= 6094.50
2.	(2) Research technicians trained in histological procedures and animal experimentation	$7200	+ " " "	= 7362.00
3.	Part time secretary and research librarian	$1200	+ " " "	= 1227.00
4.	Animal caretaker and glassware washer	$2400	+ " " "	= 2454.00
		$ 16800		$ 17137.50

Expendable supplies

1.	Animals and animal food (approximate estimation)	$ 500.00
2.	Cigarettes and other laboratory supplies (chemicals, glassware, dyes, enzymes, etc.)	800.00
		$ 1300.00

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Bioassay, carcinogenesis and tissue culture #159R3

Activated: 9/1/57
Renewed: 9/1/58
Extended to: 4/1/60
Xcf. #74
Activated: 6/1/55
Renewed: 6/1/56)
Renewed: 6/1/60

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street
New York 17, N.Y.

Committee:
Jacobson, Chm.
Reimann
Kotin
Lynch

Application for Renewal of Research Grant

Date: November 8, 1960

1. Name of Investigator: Cecilie Leuchtenberger, Ph.D.

2. Title: Senior Biologist and Cytochemist

3. Institution & Address: Children's Cancer Research Foundation
35 Binney Street
Boston 15, Mass.

Address after April 1, 1961:

Inst. f. Allgemeine Botanik
d. Eidg. Techn. Hochschule
Zuerich, Switzerland
Director, Prof. A. Frey-Wyssling

10035414M

4. Project or Subject:

The interrelation between influenza virus infections and exposure to cigarette smoke in the development of pulmonary and of bronchial lesions in mice.

(A correlated histological, cytological and cytochemical study of the tracheobronchial tree and lungs.)

5. Detailed Plan of Procedure:

The purpose of this project is the continuation and extension of the study of the sequence of events occurring in the tracheobronchial tree and lungs of mice exposed to cigarette smoke. Particular attention will be focused on the possible role of influenza virus infections in the production of bronchial and pulmonary lesions. Correlated histological, cytological and cytochemical investigations will be carried out and combined with the application of newer quantitative cytochemical techniques, such as microspectrophotometry, interference and fluorescence microscopy. It is felt that these methods will be of great help when attempting to explore the interrelationship between virus infections and exposure to cigarette smoke in the development of bronchial and pulmonary lesions in mice.

Results of previous studies done in our laboratory on the project, which are concerned with the histopathological, cytopathological and cytochemical changes in the tracheobronchial tree and lungs of mice after exposure to cigarette smoke, have been reported at National and International Congresses and have been published under the titles:

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

I. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE

Bronchitis with Atypical Epithelial Changes in Mice Exposed to Cigarette Smoke
Cancer, Vol. 11, No. 3, May-June, 1958

II. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE SEQUENCE OF EVENTS IN THE BRONCHIAL EPITHELIUM FROM MICE EXPOSED TO CIGARETTE SMOKE
Acta Union Internationale contre le Cancer, Vol. XV, Nos. 3-4, 1959

III. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE

Varying Responses of Major Bronchi to Cigarette Smoke, Absence of Bronchogenic Carcinoma after Prolonged Exposure, and Disappearance of Bronchial Lesions after Cessation of Exposure
Cancer, Vol. 13, No. 4, 1960

IV. A CORRELATED HISTOLOGICAL, CYTOLOGICAL, AND CYTOCHEMICAL STUDY OF THE TRACHEOBRONCHIAL TREE AND LUNGS OF MICE EXPOSED TO CIGARETTE SMOKE

Unaltered Incidence of Grossly Visible Adenomatous Lung Tumors in Female CF_1 Mice after Prolonged Exposure to Cigarette Smoke
Cancer, Vol. 13, No. 5, 1960

On the basis of the studies, published under I-IV, it appeared that in cases in which a harmful effect on the tracheobronchial tree has been observed after exposure of mice to cigarette smoke, the cigarette smoke may not be solely responsible, but that there may be other elements within the host which may render the tissue susceptible to smoke. The concept that the tracheobronchial tree may, indeed, need a "special conditioning" for the injurious action of the cigarette smoke is supported by our data on mice- that the difference in response and resistance of the bronchi to cigarette smoke is independent of dose and length of exposure.

1003541402

While it is impossible, at present, to relate any particular factor or factors of the host to the resistance or susceptibility of the tracheobronchial tree to cigarette smoke, the investigation of agents possibly carried by the host, which may contribute to the injury of the bronchi, would seem of great interest. Among the many possible host factors, viruses deserve special consideration - the more so since they occur with a certain frequency in mice and are capable of producing respiratory lesions.

It is well known that mice may be carriers of latent viruses which, under certain circumstances, may provoke alterations. In view of the increasing recognition of the dual role of viruses as either cytolytic agents or stimulators of cell proliferation, which may even lead to tumor formation, the investigation of viruses in combination with exposure to cigarette smoke seems to us particularly worthwhile.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

At present we are engaged in studies concerned with the question of whether a mouse adapted influenza-virus will alter the effect of exposure to cigarette smoke on the tracheobronchial tree and lungs of mice.

For this purpose, mice were divided into 5 groups as follows:

Group 1, consists of non-exposed controls

Group 2, consists of mice infected intranasally with influenza virus

Group 3, consists of mice exposed to cigarette smoke

Group 4, consists of mice exposed to cigarette smoke followed by intranasal influenza virus infection

Group 5, consists of mice infected intranasally with influenza virus followed by exposure to cigarette smoke.

In view of the importance of long range studies, mice were infected with influenza virus in such a manner that survival of these animals was assured.

During the remaining time in Boston, these mice will be sacrificed at certain intervals and all the organs necessary for this study processed for the combined morphological and cytochemical examination. This material will be taken to the Institute for Allgemeine Botanik (Director - Prof. Dr. A. Frey-Wyssling) at the Eidgen. Techn. Hochschule in Zuerich, Switzerland, where it will be further prepared in order to carry out the proposed combined morphological and cytochemical investigations at the department of cytochemistry (Prof. F. Ruch) at the Institute in Zuerich.

6. Budget Plan: * see under 10

Salaries	$ 22,500
Expendable Supplies	1,500
Permanent Equipment	------
Overhead (none)	------
Other	------
	$24,000

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7. Anticipated Duration of Work: 1 to 2 years

8. Facilities and Staff Available: The Institute for Allgemeine Botanik at the Technische Hochschule in Zuerich, Switzerland, has a special department of cytochemistry (Prof. F. Ruch) with all facilities for histo-and cytopathological work. This laboratory is also equipped with numerous modern optical instruments including special scanning microspectrophotometer and microinterferometer which will be completely at our disposal. Prof. F. Ruch, who designed some of these instruments, is greatly interested to collaborate with us in applying these special methods and instruments for the study of the chemical alterations at the single cell level after virus infections and exposure to cigarette smoke.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Explanation of Salaries:

Senior investigator	$11,000 (present salary)
Part-time Pathologist	8,000
Laboratory assistant	3,500

The $1500 will be needed for chemicals, glassware, slides, enzymes etc.

/s/ Cecilie Leuchtenberger
Director of Project

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

How related to health
Pretty far fetched
Smoke

Application For Research Grant

Date: August 16, 1954

1. Name of Investigator: Dr. Carl J. Likes

2. Title: Project Supervisor

3. Institution & Address: Virginia Institute for Scientific Research
326 N. Boulevard, Richmond 20, Virginia

4. Project or Subject:

The Title of the proposed research project is "Metabolism and Catabolism of Leaf Proteins in Tobacco (Nicotiana tabacum)". The general object of the study will be to isolate and to characterize physiochemically (a) the vacuolar, (b) the chloroplastic, and (c) the cytoplastic proteins present in tobacco leaves under varied conditions of growth, aging, and curing.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The prepapartion of the various protein fractions will be carried out as follows:

A. Vacuolar Protein. The vacuolar components of green tobacco leaves will be extracted using Chibnall's method (1), which involves cytolysis of the leaves with "used" ether-water and removal of the diluted plant juices with the aid of a hydraulic press. If feasible, the press residues will be processed to yield chloroplastic and cytoplastic protein by methods discussed below.

B. Chloroplastic Protein. The isolation of this fraction will be attempted by means of the differential centrifugal methods of Menke (4) and Granick (2). The lipid portion, consisting of chlorophyll, carotinoid pigments, etc., will be removed by extraction with ethanol and ether, or by the use of digitonin and other agents as described by Smith and Pickels (5).

C. Cytoplastic Protein. The separation of chloroplastic and cytoplastic protein has been achieved by Chibnall (loc. cit.) by filtering ground, cytolyzed leaves through thick wads of filter pulp. However, the yields have been poor, and this method will be employed only in the interest of preparing purified cytoplastic protein samples.

Director of Research

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

5. Detailed Plan of Procedure - Contd.

As an alternative approach, extraction of the total leaf proteins will also be made, and with the data obtained on the chloroplastic fraction, some information relating to the composition of the cytoplastic fraction may be obtained by difference. The total leaf protein extractions will be made with mildly alkaline buffers in the presence of ethanol and ether (Lugg and Weller (3)) or with neutral buffers supplemented by ultracentrifugation as described by Wildman et al (6).

The various protein samples will be purified by electrophoresis and by ultracentrifugation in as far as feasible and their molecular ~~wtk~~ weight, size, and shape estimated from ultracentrifugal and diffusion data.

The representativeness of the protein samples as related to the actual protein composition of the whole leaf will be checked by total nitrogen determinations (kjeldahl), Van Slyke amino nitrogen assays, and by analysis of selected amino acids (tyrosine, trytophan, etc.). The protein content of prepared samples will be estimated by trichloroacetic acid precipitation and the amino acid composition ~~determined~~ determined if feasible by chromatographic or by purely chemical methods.

It is planned to begin the program with studies on green leaves and then, depending on the progress made, to extend the investigation to harvested leaves which have been aged and cured for various periods of time.

References

1. Chibnall, A.C., Miller, E.J., Hall, D.H., and Westfall, R.G., Biochem. J. 27, 1879 (1933).

2. Granick, S., Amer. J. Bot., 25, 558 (1938); ibid, p. 561.

3. Lugg, J.W.H. and Weller, R.A., Biochem. J., 38, 408 (1944)

4. Menke, W., Z. Bot., 32, 273 (1937)

5. Smith, E. L. and Pickels, ~~E~~ E.G., J. Gen. Physiol., 24, 753 (1941)

6. Wildman, S. G., Cheo, C.C., and Bonner, James, J. Biol. Chem., 180, 985 (1949)

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan:

	Applicant	Granted
Salaries	$ 7,000.00	
Expendable Supplies	500.00	
Permanent Equipment	800.00	
Overhead	7,000.00	
Other	200.00	
Total	$15,500.00	

Date

7. Anticipated Duration of Work:

Two Years

8. Facilities and Staff Available:

With the exception of a colloid mill, hydraulic press, and a Van Slyke apparatus the major items of equipment are on hand. These include a Spinco Model E Ultracentrifuge and a Perkin-Elmer Model 38 Electrophoresis unit, X-ray diffraction and electron microscopic equipment, if required, are also available.

The addition of a plant biochemist to our present staff is also contemplated if the project is approved.

9. Additional Requirements:

Green house facilities are not available. However, an arrangement may be made with the Research Department of the American Tobacco Company, located in Richmond, to furnish green leaf material. A working agreement may be possible with the Virginia-Carolina Chemical Corporation, which has green house facilities.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The High Polymer Group at the Institute has been engaged for the past three years on a basic study of zein (corn protein) for the Virginia-Carolina Chemical Corporation, and it is felt that the experience gained in this work would be of great value in the proposed study of leaf proteins in tobacco. The group is also engaged in an ultracentrifugal study of lipoproteins in human cardiac and diabetic sera, and it may prove feasible to apply some of the techniques derived from this work to the study of the lipoproteins of the chloroplastic fraction in tobacco.

Signature

Director Carl J. Likes

/s/ Henry Leidheiser, Jr.

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Cardiovascular, pharmacology and chemistry

#281

Committee:

Drs. Bing, Chm.
Cattell
Jacobson

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

Application For Research Grant

Date: August 19, 1960

1. Name of Investigator: Esten O. Lindseth, M.D.

2. Title: Associate in Surgery (Thoracic)

3. Institution & Address: Emory University School of Medicine
Atlanta 22, Georgia

4. Project or Subject: Measurement of the differential blood flow in the mammalian lung during the acute period of smoke inhalation, using the open chest animal and the method and principle of gamma emitting radioactive graded microspheres.

5. Detailed Plan of Procedure:

A. Theory

When the blood flow through an organ, part of an organ, or extremity is to be measured, it is obviously of great interest how much the fractional blood flow through the A-V anastomoses, the A-V bridges and the tissue capillaries is of the total blood flow per unit weight of tissue. Due to the dual function of the capillaries in the lung and because the pulmonary A-V anastomotic and the tissue capillary flow ratio might be different as compared with other organs, it is of great interest to study these fractional flows in the mammalian lung under normal conditions, and in this case particularly with respect to periods of acute smoke inhalation, and during the injection of nicotine in graded concentrations. There have been several approaches made to this problem, but very few apply to the study of the pulmonary fractional flow, mainly because of limitation of methods. The methods of cast injection preparations and the perfusion with immiscible fluids through the lung principally demonstrate the existence of arteriovenous anastomoses in the lung, but the disadvantages are obvious as one cannot conclude anything about the quantity of the functional flow through these arteriovenous anastomoses.

The use of inert gases with high diffusion capacities and the use of oxygen content difference in the pulmonary artery and veins using the Fick principle obviously cannot distinguish between true arteriovenous anastomotic shunt flow with ventilation perfusion ratio of zero, and poorly ventilated alveolar capillaries with ventilation perfusion ratio below the average of the whole capillary bed. The total lung O_2 consumption of approximately 10% of total body O_2 consumption will also automatically be integrated in the results.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

We have, therefore, developed a method which has proved to be able to measure the blood flow through the arteriovenous anastomoses, the arteriovenous bridges and indirectly the capillary blood flow as the total blood flow was measured simultaneously. A known number of radioactive graded glass microspheres with an arithmetic mean diameter of 20 microns and therefore small enough to pass through the arteriovenous anastomosis, but too large to pass through the capillaries or the A-V bridges, was injected into the artery of a segment of the organ. In the beginning of the experimentation the venous outflow was collected and the microspheres within were then counted microscopically. This worked well as long as the perfusion fluid was clear, oxygenated dextran or saline, or Krebs solution but we encountered considerable difficulties in finding a method for decolorization of the venous blood when blood was used as perfusion liquid. We reasoned therefore that if the spheres were made radioactive a simple determination of the radioactivity of the venous blood would provide the information necessary to calculate the proportion of the injected beads that passed through the A-V shunts. A mean of 2 aliquots was used as the aliquot for each injection. This was with the reasonable assumption that the spheres are distributed at arterial bifurcations in the same proportion as is the blood, the fraction of injected spheres recovered was taken as a measure of the fraction of arterial blood that had passed through the A-V anastomosis.

This same principle may also be used studying the A-V bridge flow with the use of microspheres with an arithmetic mean diameter of 14 microns.

B. Method

The microspheres will be obtained from the Minnesota Mining and Manufacturing Company. They are received as a crude mixture with diameters varying from 5 to 100 microns, and with the factory information with a mean diameter of approximately 28 microns. Batches of these will have to be fractionated into homogenous samples, whose size frequency curves will be determined. The fractionation occurs by a series of multiple elutriations and timed decantations. In order to obtain microspheres from these batches with a diameter of approximately 14 microns, it is found that a Cook's Elutriator is necessary. (S. R. Cooke. U.S. Bureau Mines Reports of Invest., no. 3333, 39, 1937.) Another method is to make the microspheres oneself by a torch flame method. (K. Sollner. Ind. and Eng. Chem. 11, 48, 1939.)

The method of making the microspheres radioactive consists of subjecting the microspheres to neutron bombardment in the atomic carbon reactor at the Oak Ridge National Laboratory. Through activation analysis we found that the main radioactive source in the bombarded microspheres was sodium^{24}, which had been converted from the ordinary sodium^{23} group by neutron capture. The glass of which these spheres are made was found to contain approximately 10% sodium oxide by weight. The sodium^{24} which is mainly a neutron, gamma emittor can thus be monitored with the appropriate gamma ray detectors. The gamma emitting microspheres can thus be measured in blood or tissue without concern for self absorption. The specific gravity of the glass that the microspheres are made from is given as 2.5, but we found that over 95% have a specific gravity between 1.59 and 2.0, as they all contain some micro air bubbles. This compares more favorably with the blood specific gravity than plastic spheres with a specific gravity of 0.98.

The method can be summarized briefly as follows:

1. A small amount of the radiated microspheres in the range of 30 to 50 milligrams is suspended in a vial of $1\frac{1}{2}$ per cent gelatin or 50 per cent dextrose solution. This suspension is stirred with a magnetic stirrer to keep the spheres in suspension as close to a steady state as possible with meticulous observation in keeping the temperature constant so that aliquots with as small a variation as possible may be withdrawn.

1003541409

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

2. About 10 or 20 microliters of this suspension is drawn with a micrometer syringe into a short section of polyethylene tubing, the concentration of the spheres being such that this volume contains no more than 1,000 spheres.

3. Through a 27 gauge needle tip at the end of the polyethylene tubing, these spheres are injected into the inferior pulmonary artery on the left side supplying the left lower lobe.

4. The inferior pulmonary vein has been cannulated. The cannula is a modified Y shape plastic type, the alternate clamping of two of the trifurcations will direct the blood either back to the left atrium of the animal or into a collecting tube. The venous outflow is collected for 20 or 30 seconds, as previous experimentation has shown an occasional bead may arrive after 20 second, but no beads will arrive after 30 seconds. On the basis of calculations of the radioactivity per bead, one has found no more than three beads will arrive after a 20 second collection.

5. The glass microspheres are allowed to settle to the bottom of the tube which is then placed in a conventional well-type crystal scintillation detector with an appropriate monitoring device, so that the radioactivity of the sphere containing blood can be determined. We have shown with previous experimentation that decantation of the supernatant fluid and subsequent centrifugation of this fluid shows no radioactivity, i.e., all beads settle to the bottom of the tube permitting for gamma-scintillation detection a very constant geometry.

6. The total blood flow through the left lower lobe will then be measured directly during the phasic inflation of the lung with a known inflation pressure with and without cigarette smoke. The fraction which the radioactivity in the collected venous blood represents of the aliquot thus indicates the arteriovenous anastomotic flow and the capillary flow can thus be easily calculated.

7. The method has been fully developed and applied to several organs at institutions where the Investigator has worked previously for his Ph.D. (Department of Physiology, University of Minnesota) and pilot studies have been made in dog lungs (the University of Maryland Hospital).

8. Due to the highly controversial matter at the present time of the effect of smoke inhalation, particularly in the pulmonary circuit, it is felt highly desirable to investigate the proposed study.

9. As there is no known knowledge regarding A-V bridge flow in the mammalian lung, this would have to be investigated with microspheres with a mean diameter of 14 microns. As mentioned above, these microspheres are extremely difficult to separate with ordinary elutriation equipment, in spite of making the Z-potential optimal for bead distraction by alkalinization, blowtorch equipment in the range of $350 or a Cook elutriator will be required.

6. Budget Plan: (See attached sheet for breakdown.)

Salaries	14,178.00
Expendable Supplies	1,365.00
Permanent Equipment	3,665.50
Overhead (15%)	2,601.45
Other	1,800.00
Total	$23,609.95

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

7. Anticipated Duration of Work: 1 year

8. Facilities and Staff Available: Investigator
The above Technician
The Lab Attendant
One Thoracic Surgery Resident
Dr. Alan Horres, Ph.D., in charge of cardiopulmonary research lab.

9. Additional Requirements:

Rooms available in Surgical Research Laboratory:
(436) Large operating room, 16' x 16'3", with extension 6'20" x 20"
One room for sterile setup and autoclaving, 9'3" x 17'8"
(446) Gas determination laboratory, 16'2" x 16'4"
(456) Cardiopulmonary function laboratory 11'5½" x 18'8"
(450) Radio monitoring and equipment room, 16'9" x 17'5". (This will have to be shared with another department.)

10. Additional Information:

This work will be related to other projects in the field of differential flow in and to various organs.

We anticipate, with your support, to extend the present investigation to the differential flow in human emphysematous lungs. As smoking has been blamed as the major contributor to the development of emphysema, it would be quite pertinent to find out whether the flow pattern in the lung acutely exposed to cigarette smoke shows any similarity to the blood flow pattern in the emphysematous lung.

As this Institution has quite a few emphysema patients under treatment, we have access to a fairly large file of such patients. The extended study then would include pump perfusion studies with oxygenated blood through the excised human emphysematous lungs.

It is obvious that this method has a wide variety of applications, in the physiological and pathological states of various organs.

We have at the present time no other source of supply for this possible variety of projects.

Signature Esten Lindseth
Director of Project

Ewell E. Bessent
Treasurer and Controller

100354411

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

BUDGET PLAN:

Salaries:	Investigator	6,000	
	Social Security	144	
	Technician	4,800	
	Social Security	144	
	Dog Lab Attendant	3,000	
	Social Security	90	
			14,178
Expendable Supplies:			
	Glassware, chemicals, microspheres	825	
	30 Dogs @ $9	270	
	540 Dog days @ .50/dog/day	270	
			1,365
Permanent Equipment:			
	Elutriation equipment, excluding the Cooks elutriator	500	
	Stopwatch and stand	22	
	Brownwill infusion apparatus	560	
	Microscope, B.&L., BVB 73, and illuminator	442	
	Filar micrometer eyepiece	103	
	Glass stage micrometer disc	21.50	
	Scaler 181A, Nuclear-Chicago	830	
	Timer T-1	102	
	Scintillation Well Counter, N-C, Model DS5-5	1,035	
	Transportation	50	
			3,665.50
Other:	Cost of irradiation of microspheres per shipment $60 + $15 transport, 24 shipments	1,800	
			1,800
			$21,008.50
Overhead, at 15%			2,601.45
	TOTAL		$23,609.95

100541412

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #240

Report No. 1

Robert H. Linnell, Ph.D.
The University of Vermont

November 21, 1959

The Oxidation of Nicotine by Gaseous Oxygen:
Mechanism, Products and Kinetics

Dear Dr. Hockett:

I am enclosing a draft copy of a proposed publication on the oxidation of nicotine. I would appreciate your critical comments and suggestions.

I am quite excited about the nicotine-oxygen work as it is now at the point where real progress on mechanism can be made. I did finally receive a grant from the National Science Foundation for similar work with pyrrole. This pretty well takes care of equipment needs and leaves only the question of a fellowship for a graduate student, which would greatly expedite the work. There is another staff member here, a Ph.D. from Harvard, who has been inactive in research for several years (his wife passed away several years ago, leaving him with four children and it has been pretty difficult for him; he has recently remarried and things are looking up now), who has shown some interest in this problem and I'm hoping that he may decide to work with me. I think that I might get modest support from an individual tobacco company, but it certainly would be better to deal with the TRIC. I talked to Mr. Hanmer in September about this and he felt that TIRC should be the logical place for me to seek support. I do appreciate your help of the past and I don't want to put you in a difficult position. I would be glad to submit another request to TIRC and then if the committee takes unfavorable action I will seek other support.

I will look forward to your comments on the above items after you have had the opportunity to consider them.

Sincerely yours,

/s/ Robert H. Linnell

RL/s

P.S. I want to publish this in Arch. Biochem. & Biophys.

1003541413

Source: https://www.industrydocuments.ucsf.edu/docs/ffvm0000

FIRST DRAFT COPY

Not for publication

CONFIDENTIAL

The Oxidation of Nicotine. I. Kinetics of the Liquid Phase Reaction Near Room Temperature

Robert H. Linnell

Department of Chemistry

University of Vermont, Burlington, Vermont

Introduction

Although the biochemical degradation and oxidation of nicotine has been extensively investigated (1), the kinetics of the reaction of oxygen with nicotine has not previously been reported. Obabko (1) reports that heating tobacco containing 25-28% H_2O (in an inert N_2 atmosphere) to 70-75°C. does not deprive the tobacco of its ability to bind O_2 from air under fermentation conditions (35°C. and 75% relative humidity) although oxidase is destroyed. The heat treated tobacco absorbed O_2 intensely initially but decreased with time whereas non-heat treated tobacco reaches a maximum O_2 uptake rate after some time and then decreases. Recently Wada (2) has oxidized nicotine at 30° C. with pure O_2 and found about 20% of the nicotine oxidized in 4 weeks. He found ammonia, methyl amine, nicotinic acid, myosimine, cotinine, nicotyrine and unidentified products.

Experimental

Nicotine was vacuum distilled in a Todd Column packed with glass helixes and the fraction at 97-98° (10 mm.) taken (reflux ratio 10:1). Freshly distilled, water-clear nicotine turns yellow in a few hours when exposed to air but when scaled off in a glass ampule under vacuum it remains colorless for months. Preliminary work was done using an AMINCO Warburg apparatus at 20-40° and with various shaking rates. It was found that with N_2 in the Warburg there was no volume change, with O_2 there was a rapid uptake of gas which was roughly 5 times the uptake rate using air; rates increased with shaking speeds and increased with temperature. Nicotine acidified with HCl, H_2SO_4 and HOAc did not have any O_2 uptake activity indicating that the free base is involved in the oxidation. To obtain kinetic data an apparatus was constructed which consisted of a flat bottom flask of about 50 ml. volume fitted with a ground glass stopper for filling and a side arm which was connected to a 100 ml. gas burette containing sat. NaCl-H_2O and a 3-way stopcock for flushing the apparatus with gas. Oxygen gas at 1 atmosphere pressure was used in all experiments. The reaction flask was mounted on a shaker which had variable amplitude and speed and was immersed in a water bath regulated to $\pm$ 0.1°C. Experiments were performed at various shaking amplitudes and speed with nicotine samples of 1.00-5.00 ml. A sample size of 2.00 ml. and shaking conditions such that rates of oxygen uptake were independent of shaking for the temperature range of 15-40° were used in this study. Rates were found to decrease on aging of freshly distilled nicotine. Vacuum storage prevents aging but air contact during sample removal gradually aged the stored nicotine; this problem was solved by using Matheson Prepurified N_2 (8 ppm O_2) to flush out the nicotine storage flask after each sample removal and in this way identical rates could be obtained from the same nicotine over a period of one week.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Oxygen was bubbled into nicotine held at temperatures from 50-70°C. The nicotine rapidly turned brown and an infrared band just before 6 microns, absent in pure nicotine, increased in intensity as the experiment continued. The infrared band suggests cotinine is being formed (3). Exodation for several days and then using Frankenburg's (3) technique for isolation of cotinine indicated complex reaction products. Addition of a free radical initiator, α,α'-Azodi-isobutyronitrile (AIBN), in a similar experiment, resulted in more rapid darkening of the nicotine and similar, if not identical, reaction products. Addition of an antioxidant, 4-methyl-2,6-di-tert-butylphenol (BHT), greatly decreased rate of coloration of nicotine on oxidation and inactivated nicotine to O_2 uptake for several hours.

Several experiments were done on a burley tobacco containing 5.1-5.2% nicotine. Small samples of the tobacco were sealed in glass tubes containing N_2, air or O_2 and the tubes heated for various periods of time in a hot air oven. Nicotine was determined by the method of Cundiff (4). No change in nicotine content was found with N_2 at temperatures from 85-125°C. With O_2, the nicotine was reduced to one-half its original value in 300 hours at 85°, 24 hours at 110° and 3 hours at 125°.

Results and Discussion

In Figure 1 typical plots of volume O_2 used vs time are shown. Table 1 tabulates all the results where rates are those from the linear part of the reaction. An Arhenius plot of the maximum rates of table 1 yields an activation energy of 6.81 kcal (least squares). This is a low activation energy compared to the uncatalyzed oxidation of tetralin from 65-130°C., where the overall activation energy is 14.9 $\pm$ 0.6 kcal. (5). If the linear rates are extrapolated back to zero oxygen uptake, and this time called the induction period $t_{ind.}$, a plot of log $(1/t_{ind.})$ vs 1/T would yield an activation energy for the removal of inhibitor. A value of 14.5 kcal. is found. The induction period could be due to formation and decomposition of a nicotine hydroperoxide but it is premature to associate an activation energy with this proposed mechanism. A small evolution of gas in the initial periods of lower temperature rate studies is probably due to decreased gas solubility in the nicotine at lower temperatures.

In the experiments reported here, 2.00 ml. nicotine, at room temp., was used, corresponding to 1.88×10^{-2} moles. For the consumption of one mole O_2 per mole nicotine we would therefore require 421 ml. O_2 at STP. Most experiments were run to a total O_2 consumption of 20-30 ml. (STP) and inhibition (deviation from linear rate) was noted at about 15 ml. (STP) O_2 consumption. The low O_2/nicotine mole ratio at which inhibition is found (about 0.03) together with inhibition by BHT and catalysis by AIBN provides strong evidence of the free-radical nature of the oxidation. This indicates nicotine oxidation follows the general mechanism of olefin oxidations (6) and a nicotine hydroperoxide could be expected as an intermediate. This hydroperoxide would be unstable at room temperature, yielding H_2O_2 (which could form nicotine N-oxide), cotinine, H_2O, and radicals which could form polymers. Further work is underway to isolate the proposed hydroperoxide and provide more details on the mechanism of the reaction.

Acknowledgement

The author thanks the Tobacco Industry Research Committee who provided financial assistance making this work possible.

1003541415

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

References

1. McKennis, H. Jr., Turnbull, L.B., Wingfield, H.N. Jr., and Dewey, L.J., J. Am. Chem. Soc. 80 1634 (1958); ibid 6597.

2. Wada, E., Kisaki, T. and Saito, K., Arch. Biochem. and Biophys. 79 124 (1959).

3. Frankenburg, W. G. and A.A. Vaitekunas, J. Am. Chem. Soc. 79 149 (1957).

4. Cundiff, R.H. and Markunas, P.C., Anal. Chem. 27 1950 (1955).

5. George, P., Rideal, E.K. and Robertson, A., Proc. Roy. Soc. A 185 288 (1946).

6. Bateman, L., Quart. Rev. 8 147 (1954).

1003541416

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Table I

Linear Rates of Oxygen Absorbed by Liquid Nicotine

Run	Temp. °C.	Ml. O_2 (STP) x 10^3 sec x ml. nicotine^{-1}	Induction period sec x 10^{-3}	Remarks
IX-20	15	1.08	5.60	Nicotine stored 24 hrs. under N_2.
XIV-24	20	1.35	2.46	Nicotine stored 10 days under N_2.
V-18	25	1.61	1.60	Nicotine fresh from vacuum dist. No storage.
VII-19	25	1.61	2.12	Nicotine stored 24 hrs. under N_2.
XIII-23	25	1.59	2.22	Nicotine stored 10 days under N_2.
XV-25	25	1.47	1.98	Nicotine stored 14 days under N_2.
XVI-25	25	1.54	1.70	Nicotine stored 14 days under N_2.
XVII-26	25	----	29	Added 50 mg. BHT
XII-22	30	1.73	1.58	Nicotine stored 4 days under N_2.
VIII-20	35	2.09	0.86	Nicotine stored 24 hrs. under N_2.
X-21	35	2.10	0.88	Nicotine stored 3 days under N_2.
XI-21	40	2.34	0.64	Nicotine stored 4 days under N_2.
II-16	25	1.21	2.10	Nicotine yellow from storage one month in poor vacuum.
III-16	25	0.80	----	Reused nicotine from II-16 immediately.
IV-16	25	0.18	----	Reused nicotine from III-16 24 hr. later.

1003541417

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

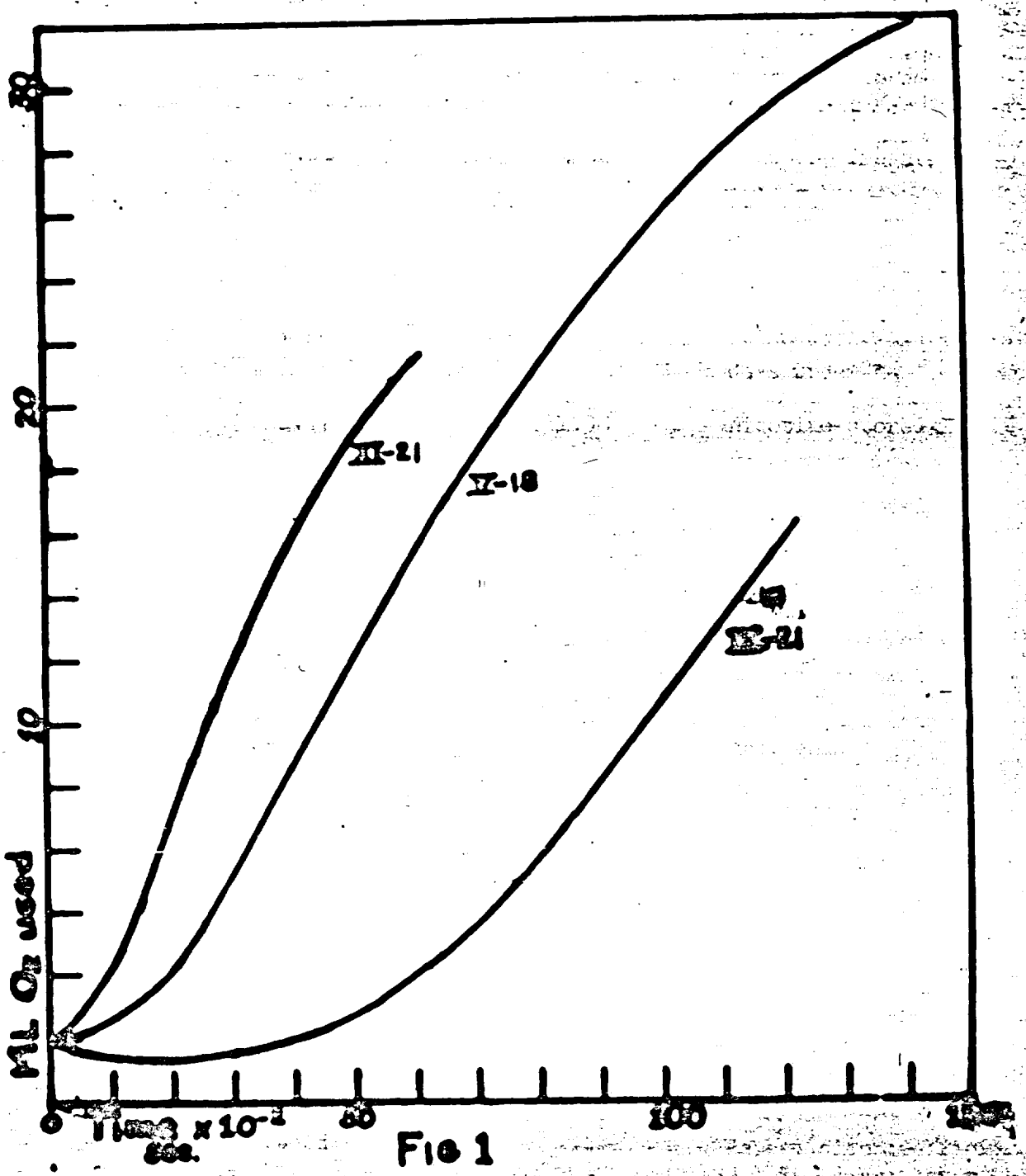

Fig 1

1003541418

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Committee:
Dr. Jacobson
Dr. Wilson
Dr. Little
(Dr. Parmele)

#264

Cf. #240
Activated 7/1/1959
for 3 months.

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street, New York 17, N.Y.

Application For Research Grant

Date: February 5, 1960

1. Name of Investigator: Robert H. Linnell, Ph.D.

2. Title: Associate Professor of Chemistry

3. Institution & Address: University of Vermont, Burlington, Vermont

(1) Possible application to aging
(2) application to Nicotine pathway
RR

4. Project or Subject: The Autoxidation of Nicotine

5. Detailed Plan of Procedure:

The rate of oxygen uptake for solutions of nicotine will be determined in the presence of an initiator, such as Azodi-iso-butyronitrile and in the presence or absence of several inhibitors, including known nicotine degradation products (cotinine and its hydrolyzed form γ-(3-pyridyl) - γ- methylamino butyric acid). By using various temperatures a detailed kinetic scheme should be worked out. We will attempt to prepare peroxy compounds of nicotine by using lower temperatures (dry ice and higher) and initiation by, a) ultrasonics, b) Gamma rays from Co-60. Infrared will be used to seek the hydroperoxide of nicotine and study its conversion to cotinine. We find that the analysis of cotinine by infrared is feasible so this method will be used. A summary of our work is enclosed with this request.

possible repetition of McKennis work at M.C.V.

6. Budget Plan:

Salaries		$3,500.00
Expendable Supplies		532.60
Permanent Equipment		600.00
Overhead	(15%)	717.40
Other (Travel)		150.00
Total		$5,500.00

1003541419

7. Anticipated Duration of Work: The funds requested are to cover the period June 15, 1960 - September 15, 1961.

8. Facilities and Staff Available: The major part of the salary request ($3150.00) is to cover a research fellowship for the stated period. This fellowship will allow a student to devote his major efforts to this research and to obtain an M.S. degree in chemistry with this work as his thesis. The chemistry department has the necessary laboratory

Source: https://www.industrydocuments.ucsf.edu/docs/tfvh0000

facilities to carry out this work including an infrared spectrometer, Beckman DU and DK for visible and ultraviolet work, etc. Equipment for this kinetic work was constructed during the summer of 1959 thanks to a TIRC grant. Ultrasonic equipment has recently been acquired. A cobalt-60 source is available at the university.

9. Additional Requirements: Some additional infrared cells and sampling equipment as well as replacement parts for the infrared are required. Modest expendable supplies cover the cost of chemicals, glassware and miscellaneous.

10. Additional Information (Including relation of work to other projects and other sources of supply):

A study of the reaction of oxygen with pyrrole is now underway under NSF sponsorship. The mechanism of this reaction is under investigation by a kinetics study, both in the presence and absence of initiators and antioxidants. We are interested in isolating peroxy intermediates believed to be present. The initiation of the reaction in the absence of a free-radical source is under study as some evidence indicates the possibility of oxygen complexes although the existance of a magnetic perturbation (by O_2) causing a forbidden singlet-triplet transition can not be ruled out.

We believe that the reaction of oxygen with nicotine is of importance in tobacco technology and that non-enzymatic, and non-bacterial reaction accounts for an important part of the fate of nicotine in tobacco processing. More information on this physical-chemical reaction is therefore needed.

/s./ Robert H. Linnell
Director of Project

/s./ John T. Fey, President
Business Officer of the Institution

1003541420

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#219

Application For Research Grant

Date:

November 20, 1953

1. Name of Investigator:

Robert H. Linnell, Ph.D.

2. Title:

Associate Professor of Chemistry

3. Institution & Address:

University of Vermont
Burlington, Vermont

4. Project or Subject:

The Oxidation of Nicotine by Gaseous Oxygen: Mechanism, Products and Kinetics

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Previous work by the applicant (unpublished) indicates that cotinine (N-methyl-2-(3-pyridyl)-5-pyrrolidone) is a major product of the O_2 oxidation of nicotine at temperatures near ambient (see Frankenburg, J.A.C.S. 72 149 (1957)). The reaction may proceed through a nicotine-oxygen complex (pi-electron complex) as is probably the case with pyrrole (see Linnell, Arch. Biochem. and Biophys. 57 264 (1955)). Low temperature nmr ultraviolet studies will be used to study the nicotine-oxygen complex. Infrared provides a convenient means for analysis of cotinine in the presence of excess nicotine (see Frankenburg above and Eddy and Eisner, Anal. Chem. 26 1428 (1954)) as well as detection and analysis of other nicotine derivatives. A vacuum system will be built to study oxygen uptake and determine if all the oxygen forms cotinine. The Kinetics of the reaction with oxygen will be followed by at a series of temperatures, near ambient temperatures. Pure cotinine will be prepared and used as a standard for infrared analysis; stability of cotinine to oxygen will also be investigated. Later studies are contemplated at higher temperatures where it is to be expected that complexities of mechanism and products will be found.

1003541421

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	5,500.00
Expendable Supplies	1,500.00
Permanent Equipment	3,200.00
Overhead (16%)	2,220.00
Other (travel-publication)	400.00
Total	14,820.00

7. Anticipated Duration of Work: 18 months

8. Facilities and Staff Available: A Perkins-Elmer model 112 infrared instrument with both LiF and NaCl optics and associated equipment is available. Glass blowing and conventional laboratory facilities are quite adequate for this study. The chemistry department has a strong M.S. program (A.C.S. approved) and a research fellowship for 15 months (two semesters and two summers) is contemplated as part of the salary requirement; the project director accounts for the balance of salary requirements, to be free of summer teaching and work full time on this project during the summer of 1959. The project is currently under way as a senior thesis.

9. Additional Requirements:

Expendable supplies cover requirements for pyrex glass (stopcocks, standard taper joints, etc.), chemicals, dry ice and liquid air, thermostat equipment, infrared supplies, safety supplies, etc. Under permanent equipment a vacuum pump, special fume hood (because of highly toxic nature of nicotine) and ultraviolet spectral equipment is needed.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The project director has his B.S. and M.S. (chemistry) from the Univ. of N.H. and his Ph.D. under Dr. W.A. Noyes at the Univ. of Rochester, and has worked with the physical-organic chemistry of N-heterocyclics (Photochemistry: Pyridine-Acetone, J.A.C.S. 73 3986 (1951); Pyridine-Pyrrole H-Bonding, J. Chem. Phys. 21 179 (1953) and ibid. 23 93 (1955); Anabasine, J.A.C.S. 76 1391 (1954); Polarographic Studies on Bipyridines, J.A.C.S. 77 6207 (1955); Tetramethyl bipyridine, J. Org. Chem. 22 1691 (1957); other publication in process on The Reaction of Sodium with Pyridine, Pyrolysis of Pyridine, 2,3-Diquinoline, U.V. of N=C-C=C compounds in H_2SO_4.) Currently H-Bonding studies are underway with Pyrrole and various pyridines, using infrared and a series of temperatures so as to evaluate ΔF, ΔH and ΔS. This work is supported by Research Corporation.

(continued next page)

Signature /s/ Robert H. Linnell
Director of Project

/s/ Howard M. Smith, Jr.
Secy. Division of Research in the Physical Sciences

/s/ L. R. Kisley
Business Officer of the Institution

1003541422

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Recent statistical studies have linked certain types of tobacco use with health problems, in a cause and effect relationship. These studies suggest a difference in cigarette, cigar and pipe tobacco use. A great deal of work must be done to clarify and explain these statistics. It is interesting to note, however, that cigar tobacco is treated by a fermentation process during which time nicotine greatly decreases and some cotinine is formed. The physiological actions of cotinine have not been studied. There may be a relationship here to the health problem.

Obtaining information on nicotine oxidation products and kinetics would be a step in the direction of further understanding of these complex problems.

1003541423

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#240

(Cf. #219 & 219A
denied)

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

Application For Research Grant

Date: July 1, 1959

1. Name of Investigator: Robert H. Linnell

2. Title: Associate Professor of Chemistry

3. Institution & Address: University of Vermont
Burlington, Vermont

4. Project or Subject: The Oxidation of Nicotine by Gaseous Oxygen: Mechanism, Products and Kinetics

5. Detailed Plan of Procedure: Previous work by the applicant (unpublished) indicates that cotinine ((N-methyl-2-(3-pyridyl)-5-pyrrolidone)) is a major product of the O_2 oxidation of nicotine at temperatures near ambient ((see Frankenburg, J.A.C.S. 79 149 (1957))). The reaction may proceed through a nicotine-oxygen complex (pi-electron complex) as is probably the case with pyrrole ((see Linnell, Arch. Biochem. and Biophys. 57 264 (1955))). Low temperature ultraviolet studies will be used to study the nicotine-oxygen complex. Infrared provides a convenient means for analysis of cotinine in the presence of excess nicotine ((See Frankenburg above and Eddy and Eisner, Anal. Chem. 26 1428 (1954))) as well as detection and analysis of other nicotine derivatives. A vacuum system will be built to study oxygen uptake. The Kinetics of the reaction with oxygen will be followed at a series of temperatures, near ambient temperatures. Work now in progress has shown that the oxidation of nicotine at room temperature with air is a free radical reaction which can be inhibited by antioxidants (hindered phenols, "radical traps") and accelerated by typical free radical sources such as AIBN. Reaction products are therefore complex and chromatography will be used to establish the various products.

6. Budget Plan:

Salaries	1575.00
Expendable Supplies	------
Permanent Equipment	400.00
Overhead 15%	325.00
Other (travel)	200.00
Total	$2500.00

7. Anticipated Duration of Work: This grant will be used for the summer of 1959 with other support expected in the fall from the National Science Foundation.

1003541424

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

8. Facilities and Staff Available: A Perkins-Elmer model 112 infrared instrument with both LiF and NaCl optics and associated equipment is available. Glass blowing and conventional laboratory facilities are quite adequate for this study.

9. Additional Requirements: The permanent equipment item is for a thermostat bath, shaking equipment and associated apparatus to measure rates of O_2 uptake in nicotine solutions at a series of temperatures.

10. Additional Information (Including relation of work to other projects and other sources of supply): The project director has his B.S. and M.S. (chemistry) from the Univ. of New Hampshire, and his Ph.D. under Dr. W. A. Noyes at the Univ. of Rochester, and has worked with the physical-organic chemistry of N-heterocyclics (Photo-chemistry: Pyridine-Acetone, J.A.C.S. 73 3986 (1951); Pyridine-Pyrrole H-Bonding, J. Chem. Phys. 21 179 (1953) and ibid. 23 93 (1955); Anabasine, J.A.C.S. 76 1391 (1954); Polarographic Studies on Bi-pyridines, J.A.C.S. 77 6207 (1955); Tetramethyl bipyridine, J. Org. Chem. 22 1691 (1957); other publication in process on The Reaction of Sodium with Pyridine, Pyrolysis of Pyridine, 2,3-Biquinoline, U.V. of N=C-C=N compounds in H_2SO_4.) Currently H-Bonding studies are underway with Pyrrole and various pyridines, using infrared and a series of temperatures so as to evaluate $\Delta F, \Delta H$ and ΔS. This work is supported by Research Corporation.

Obtaining information on nicotine oxidation products and Kinetics would be a step in the direction of further understanding of the complex problems in tobacco technology and in questions involving health.

Signature /s/ Robert H. Linnell
Director of Project

/s/ George N. Clerkin, Treasurer

/s/ Howard M. Smith, Jr.
Sec'y. Division of Research
in the Physical Sciences

10035441425

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6 Budget Plan per year

Salaries 6300.00
Expendable Supplies 1200.00
Application For Research Grant 300.00
Overhead 600.00
Other 900.00
Date: Total 9300.00

August 4, 1954

1. Name of Investigator of Work: 2- 3 years.
Otto E. Lobstein, Ph. D.

2. Title: ies and Staff Available: Two Ph. D. biochemists, one graduate student,
Director of Research [illegible]
assistants, and technicians.
Library and office staff.

3. Institution & Address: Chem-Tech Laboratories, 235½ So. Robertson Blfd.
Completely equipped chemical laboratory with
optical instruments, Fluorescence microscope, etc.
Beverly Hills, California
Animal quarters, cages, surgical equipment, etc.

4. Project or Subject: The effect of enzymes on the growth of lymphosarcoma.

5. Additional Requirements:
I. The role of lysozyme.
II. The role of other enzymes, proteolytic, mucolytic, and others.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Work of similar nature has been carried on in this laboratory for the last two years, therefore requirements for personnel equipment is at a minimum. Previous study in this laboratory has demonstrated:

(1) Lysozyme has a statistically significant effect on the growth of Gardner's lymphosarcoma (6C3HED) in so far as it will arrest and heal a young tumor.
(2) The survival time of lysozyme injected mice is thus significantly increased.
(3) This effect is less favourable on old tumors than on young ones.
(4) The enzyme (lysozyme) concentration does not seem to play a role in the concentration rage tested.
(5) Histological examinations show a difference in treated and untreated tumor.
(6) A healed mouse will not take an additional transplant of the tumor.

Future work:

(1) To investigate further the above findings of possible antibody formation (point 6 above) with an eye on the possibility of mouseblood fractionation to separate the antibody concerned.
(2) To investigate the effect of other proteolytic enzymes, and other enzymes, or mixtures thereof with and without lysozyme on 6C3HED lymphosarcoma. This with a view on the pH of the tumor and adjusting the enzyme mixture such as to have an optimum activity at the pH of the tumor.

Business Officer of Institution

100354426

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan: per year.

Salaries	4500.00
Expendable Supplies	1700.00
Permanent Equipment	200.00
Overhead	600.00
Other	500.00
Total	7500.00

7. Anticipated Duration of Work: 2- 5 years.

8. Facilities and Staff Available:

Two Ph. D. biochemists, one bacteriologist, one M. D. pathologist, three undergraduate assistants, and technicians.

Library and office staff.

Completely equipped chemical laboratory with optical instruments such as Beckman Model DU with UV and Flame attachments, Fluorometric photometer, etc.

Animal quarters, cages, surgical equipment, etc.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Work ofsimilar nature has been in progress for the last two years, therefore requirements for permanent equipment is at a minimum. This work has been reported in part at the meetings of the Amer. Assoc. for the Adv. of Science, Santa Barbara, California in 1953 (June); the Amer. Assoc. of Clin. Chemists, Vet. Adm. Hosp., Los Angeles, Cal., in May 1954; the joint meetings of the Council of American Biosnalysts and Calif. Assoc. of Clin. Labs., Yosemite Natl. Park, Calif., in May 1954. A paper will be presented at the September 1954 meetings of the Amer. Chemical Society in New York. Portions of the work have been accepted for publication in "The Clinical Chemist" and "American Journal of Digestive Diseases", they will appear in the fall 1954 issues of these journals.

Signature
Director of Projects

Business Officer of the Institution
Emma Sechooler

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000.

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

122

6. Budget Plan
1. Salaries
Application for Research Grant
Overhead
Other

Date: December 29, 1955

1. Name of Investigator: Francis C. Lowell, M.D. (1)
William Frankbin, M.D. (2)

2. Title: (1) Member, Evans Memorial, Massachusetts Memorial Hospitals
(2) Associate Professor of Medicine, Boston University School of Medicine

3. Institution & Address: Evans Memorial
65 East Newton Street
Boston 18, Massachusetts

4. Project or Subject: To determine in the course of the next 1½ or 2 years the incidence in an industrial population of chronic pulmonary disease (especially emphysema and asthma), and its relation to occupation, smoking and past history of certain other diseases.

* No additional requirements for this study are anticipated at this time.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): Significant pulmonary disease, often unrecognized by the subject, may exist in the absence of physical and x-ray findings. This is especially true for bronchial asthma and pulmonary emphysema. The associated functional abnormality in these conditions as well as restrictive disease may be readily detected with the expirogram, a kymographic tracing of a maximal forced expiration (1). The procedure, done before and after the administration of a bronchodilator drug, requires little time and a minimum amount of instruction for the subject. We have arranged to carry out this study in an industrial plant in this vicinity which employs approximately 2,500 persons, and have already obtained the required apparatus. Each subject is to fill out, with assistance if necessary, a detailed questionnaire* which includes a section on occupation and smoking as well as a section on respiratory, cardiac and gastrointestinal symptoms. All the above information and the test of pulmonary function will be gathered in conjunction with an annual physical examination which includes urinalysis, routine examination of the blood and x-ray of the chest. This study is already under way. We are applying for funds to make possible proper execution of the test of pulmonary function, accumulation of complete data and detailed analysis of the material.

10. Additional information, including relation of work to other projects and other

statistical analysis of data.

(1) Clinical value of a tracing of forced expiration (expirogram). I. Pulmonary function. Franklin, W., Michelson, A.D., Lowell F.C. and Schiller, I.W. N. Eng. J. Med, 253: 799-808, (Nov. 10) 1955.

s/ Francis C. Lowell
Signature

* Prepared with the assistance of Mr. John Alman, Director of Statistical Research Services, Boston University

1003541428

s/ Philip D. Bonnet, M.D.
Business Officer of the Institution
Administrator

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO
350 FIFTH

6. Budget Plan:

Salaries		$6,000
Expendable Supplies		1,000
Applicat Permanent Equipment	(10%)	900
Overhead		2,000
Other		
Total		$9,900

Date

7. Anticipated Duration of Work: 1½ to 2 years

8. Facilities and Staff Available: Facilities include the Medical Dept. of an industrial plant employing approximately 2,500 persons. Apparatus for carrying out the expirograms has been obtained. Analysis of the data is to be done by the Statistical Research Services of Boston University. The Medical Dept. of the industrial plant is staffed by 2 or more physicians, 5 nurses and 2 technicians. The request for salaries mentioned under (6) does not include personnel already employed in the Medical Department. One of us (Wm. Franklin, M.D.) is in regular attendance twice weekly and is in a position to supervise the performance of the tests and the collection of data. In addition, secretarial, technical and laboratory facilities are available in the Evans Memorial for special procedures as they may arise.

9. Additional Requirements:

A No additional requirements for this study are anticipated at this time.

10. Additional Information (Including relation of work to other projects and other sources of supply): The plan described is an extension of a continuing study of obstructive pulmonary disease which has been in progress in this division of the Evans Memorial for a number of years. These researches have been supported by the Evans Memorial and in addition by grants from the U.S. Public Health Service and from Pharmaceutical Companies. The current study described herein is, being supported in part by the Evans Memorial and space, some technical help and certain supplies have been provided by the industrial plant concerned. The apparatus has been donated by Warren E. Collins Co. of Boston.

Estimated Funds Needed:

Technician.....3,000
Recorder of data.....3,000
Statistical analysis of data2,000
Equipment and supplies.....1,000

9,000

s/ Francis C. Lowell

Signature

Director of Project

* Prepared with the assistance of Dr. John Ahlquist, Director of Statistical Research Services, Boston University

s/ Philip D. Bonnet, M.D.

Business Officer of the Institution

Administrator

1003541429

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

1003541430

Funds Needed

	3,000
data	3,000
analysis of data	2,000
and supplies	1,000
	9,000

a/ Francis C. Lowell

Salaries	56,000
Expendable Supplies	1,000
Permanent Equipment	
Overhead	900
Other	2,000
Total	59,900

(10%)

ated Duration of Work: 1½ to 2 years

s and Staff Available: Facilities include the Medical Dept. of an industrial plant approximately 2,300 persons.

for carrying out the explorations has been obtained, analysis of the data is to the Statistical Research Service of Boston University. The Medical Dept. of rial plant is staffed by 2 or more physicians, 3 nurses and 2 technicians. The r available mentioned under (6) does not include personnel already employed in l Department. One of us (Wm. Franklin, M.D.) is in regular attendance twice ld in addition, secretarial, technical and laboratory facilities are available in Material for special procedures as they may arise.

tional requirements for this study are anticipated at this time.

tional information (including relation of work to other projects and other sources of support): The plan described ation of a continuing study of obstructive pulmonary disease which has been in h this division of the Evans Memorial for a number of years. These researches supported by the Evans Memorial and in addition by grants from the U.S. Public vice and from Pharmaceutical Companies. The current study described herein is orted in part by the Evans Memorial and space, some technical help and certain ave been provided by the industrial plant concerned. The apparatus has been Warren E. Collins Co. of Boston.

Source: https://www.industrydocuments.ucsf.edu/docs/mva0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: October 15, 1954

1. Name of Investigator: **Aldo A. Luisada, M.D.**

2. Title: **Associate Professor of Medicine of The Chicago Medical School and Director, Division of Cardiology, The Chicago Medical School.**

3. Institution & Address: **The Chicago Medical School**
710 South Wolcott Avenue
Chicago 12, Illinois

4. Project or Subject: **Action of Products of Combustion of Tobacco Leaves on the Circulation**

has it? cause + effect

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

It has been proven that cigarette smokers have a higher percentage of coronary heart disease than either non-smokers or pipe or cigar smokers. This would point out to a specific action of products which are contained in the smoke of cigarettes and not to a general effect of nicotine.

why?

We would like to study on humans and animals the effect of several alkaloids which have been demonstrated in the smoke of cigarettes. The average leaves of Kentucky tobacco contains about 4% of alkaloids. Following their extraction, 95% of the total alkaloid extract is made of nicotine while 2% is a mixture of nor-nicotine and l-anabasine; 0.5% is nicotimine; 0.1% is nicotelline; about 10 other, less well known, alkaloids make up the balance. From what is known, l-anabasine is the most toxic of these alkaloids.

Need Lit. Source? info

Tobacco smoke contains some alkaloids of the tobacco leaf (mostly nicotine) and eight other alkaloids which are the result of the process of combustion. Only myosmine has been studied among the latter.

(Continued on attached page)

Signature /S/
Director of Project
Aldo A. Luisada, M.D.

/S/
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

1003541431

Continued

We plan to isolate chemically the various alkaloids of cigarette smoke and test them in the following ways:

(1) Human experiments on volunteers. Electrocardiograms, ballistocardiograms, and plethysmograms before and after smoking the first cigarette after a period of 3 days abstinence from smoking.

(This would give a standard type of reaction which could be compared with those of experimental animals).

(2) Comparison of the intravenous and intrabronchial (aerosol) administration of the various fractions of cigarette smoke in dogs.

(a) Intact, anesthesized animals (electrocardiogram, blood pressure, ballistocardiogram, plethysmogram).

(b) Heart-lung preparation (electrocardiogram, blood pressure).

1003541432

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan: TOBACCO INDUSTRY
350 FIFTH AVENUE

Salaries	7,400.00
Expendable Supplies	1,500.00
Permanent Equipment	500.00
Overhead	940.00
Other	
Total	$10,340.00

Date December 15, 1954

7. Anticipated Duration of Work: Two years

8. Facilities and Staff Available: The staff of the Division of Cardiology, including the Director and 6 full-time Research Assistants, will take part to this study. The Chemical extraction will be done by Dr. J. Sice, in the Division of Oncology of this School. Dr. Sice, Assistant Professor of Pharmacology, will also be available in the capacity of consultant.

The Technical facilities of the Division of Cardiology (including electrocardiographs, phonocardiograph, ballistocardiograph, plethysmograph, and pressure recorders) are available.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

If any specific alkaloid is proven to be the cause of coronary or peripheral vasoconstriction, it is anticipated that different methods of preparation of the tobacco leaves might exclude it from the finished product, thereby removing the main cause of morbidity in regard to the heart and vessels. It is also possible that certain drugs may act as antidotes or antagonist and might be mixed with the tobacco, thereby neutralizing the noxious effects.

Signature /S/
Director of Project
Aldo A. Luisada, M.D.

/S/
Business Officer of the Institution
Henry E. Mitchell

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Committee:
Kotin, Chairman
Reimann
Jacobson
(Lynch)

#276

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street New York 17, N.Y.

Application for Research Grant

Date: May 11, 1960

1. Name of Investigator: Kenneth M. Lynch, M.D.
Forde A. McIver, M.D.

2. Title: President and Professor of Pathology
Assistant Professor in Pathology

3. Institution & Address: Medical College of South Carolina
16 Lucas Street
Charleston, South Carolina

4. Project or Subject: Environmental factors and pulmonary disease.
I. Asbestos dust.

5. Detailed Plan of Procedure:

To continue a series of studies begun in 1948 related to industrial dusts and pulmonary tumors. In the future it is intended that other animals and other industrial and environmental dusts should be used, but the present outline is concerned primarily with the study of dogs subjected to asbestos dust and comparison with older dogs supplied by local veterinarians.

At the present time there are nine dogs which have been subjected to exposure with asbestos dust for periods up to six years. Support from another source (U.S.P.H.S.) has terminated, but nine animals are still living and in good condition. Periodic x-rays are available. Furthermore, since the study was initiated a great deal of work has been done in the study of the respiratory trees of smokers and non-smokers and it now seems advisable to study the lungs of our animals with similar techniques. The lungs of five previous animals are suitable for such study as well as the nine presently living animals. Lesser portions of lung are available on 20 other dogs previously examined. In the past, lungs from non-dusted dogs have been supplied by local veterinarians. In the proposed study additional control lungs will be obtained and studied with a similar technique and, of greatest importance, we should now do lobectomies on dogs at various stages of exposure in order to determine how long marked atypicalities may persist without malignant change. Two dogs with definite atypical changes should be selected as a pilot study and removed from exposure after lobectomy to see if regression of atypical changes does occur.

In order to continue these studies it will be advisable to redesign the dusting apparatus in such a way that more effective dusting can be done and so that the equipment will be suitable for extension of the study at a later time.

1003541434

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

*See Attached Sheet.

	1st yr.	2nd & 3rd yrs.
Salaries	4,940.	5,240.
Expendable Supplies	1,180.	1,700.
Permanent Equipment	2,200.	300.
Overhead (15% of total)**	1,293.	1,116.
Other	300.	200.
Total	$9,913.	$8,556./yr.

TOTAL (3 years) $27,025.

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available:

K. M. Lynch, M.D. and Forde A. McIver, M.D. (Principal Investigators, no salary.) Animal caretaker (part time), medical student assistant (part time), and dust counting and tissue technician. The facilities of a well-equipped Department of Pathology are available.

9. Additional Requirements: None

10. Additional Information:

The first part of this study was related to the formation of pulmonary tumors in mice and the results were negative except for the possible increased numbers of tumors in susceptible mice (Pulmonary tumors in mice exposed to asbestos dust, A.M.A. Arch. Indust. Health, 1957, 15:207). More recent studies on dogs show prominent fibrosis and striking atypical epithelial changes (to be published). It is the finding of these changes after very long term exposure which convinces us that the living dogs must not be sacrificed at the present time. We may be on the verge of malignant neoplastic formation. On the other hand, proposed studies may show that such remarkable changes may persist for many years without progressing to cancer, or that regression may occur.

Forde A. McIver
Signature Director of Project

L. W. Michaelis
Business Officer of Institution

**-Note by RCH: In several similar cases overhead allowances have been limited to 15% of budget exclusive of permanent equipment. In the present case, elimination of the $2,200. item from calculation would show $960. for overhead or total of $9,580.

10035441435

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Breakdown of "Budget Plan" for first year:

Salaries:	
Animal caretaker (part time)	1,040.
Medical student (part time)	600.
Dust counting and tissue technician	3,300.
	$4,940.
Expendible Supplies:	
Animal colony and veterinary care	780.
Photographic supplies	200.
Dust and technical supplies	200.
	$1,180.
Permanent Equipment:	
Improvements on present animal cages	500.
Dust-proof partition in dust room	500.
Dusting Equipment (blowers, dryers and conditioners)	1,200.
	$2,200.
Overhead:	
15% (See ** note on page 2)	$1,293.
Other:	
Travel: to visit Johns-Manville to discuss design of dusting equipment	150.
Travel: to American Association for Cancer Research for information on related studies	150.
	$300.

1003541436

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

STANDARD B & P "NOTEAR"

STANDARD B & P "NOTEAR"

1003541437

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

#184

Application For Research Grant

Date:

October 23rd, 1957

1. Name of Investigator:

Jack G. Makari, M.D., Sc.M., D.T.M. & H.

2. Title:

Director of Research

3. Institution & Address:

Muhlenberg Hospital
Plainfield, New Jersey

4. Project or Subject:

Studies on Cigarette Smoke Condensates by Immunologic Methods.

I. Basic Immunologic Studies
II. Immuno-epidemiologic Studies
III. Immuno-pathologic Studies

1003541438

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

I. BACKGROUND

Using the Schultz-Dale Method, I was able to detect what appears to be a specific antigen in sera of patients with carcinoma. Further work with Dr. P. Kragg, Ass't. Dir. of the Statens Serum Institute and Head of the World Health Organization Serology Center in Copenhagen, is providing further evidence of the antigen-antibody nature of this reaction in cancer.

Preliminary experiments with unknown sera (Makari unpublished work) have shown that this approach could be used not only for the detection of carcinoma antigen in the serum, but also for the detection of a carcinogen, methyl-cholanthrene, in the serum and in the tissues. Of particular interest was the high percentage of positive tests to the carcinogen (47%) in the apparently healthy individuals tested. Now that "Composite Cigarette Smoke Condensate" (C.C.S.C.) can be produced according to specifications acceptable to the Scientific Advisory Board of the Tobacco Industry Research Committee, and is available in its acidic, basic, and neutral fractions, it readily lends itself to study by such sensitive immunologic methods as the Schultz-Dale Reaction.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

5. (Cont'd.)

II. METHOD

Details of the method with preliminary results in cancer are found in the British Medical Journal, Nov. 26th, 1955, vol. ii, p. 1291 (See reprint). This approach has been carefully evaluated in an annotation in the British Medical Journal (See enclosed).

III. PLAN

A Basic Immunologic Studies

These studies will be primarily concerned with the antigenicity of the C.C.S.C. and its fractions using the Schultz-Dale reaction.

An attempt will be made to study antigenic relationships between C.C.S.C. and (1) carcinoma antigen (2) methyl cholanthrene (carcinogen) and (3) "atmospheric smog condensate" (if obtainable).

B Immuno-epidemiologic Studies

These studies will explore further the significance of high carcinogen levels in the sera of some so called "healthy individuals" and of individuals with cancer as revealed by the Schultz-Dale reaction.

An attempt will be made to correlate a high carcinogen level in the serum of these individuals with such factors as (1) smoking, (2) atmospheric pollution, (3) food habits (dyes, etc.), (4) exposure to radiations, and (5) other factors.

C Immuno-pathologic Studies

These studies will attempt to detect antigens related to C.C.S.C., carcinoma, and methyl cholanthrene in lung tissue specimens obtained at autopsy or operation. Further correlation with factors listed in (B) will be made especially in relationship to smoking habits in otherwise comparable groups.

QUESTIONS TO BE ANSWERED

1. Does the C.C.S.C. have antigenicity in common with methyl cholanthrene or carcinoma antigen? If so, which fraction is involved?

2. What is the significance of high carcinogen levels in so called "healthy individuals"? Could it be that "harmful effects of smoking", if any, are found only in those cases with high carcinogen levels in the blood and tissues to begin with? Does smoking act as a co-carcinogen? Are persons with high carcinogen levels "candidates for lung cancer"?

1003541439

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

	Total 3 years
Salaries	$24,000
Expendable Supplies	9,000
Permanent Equipment	7,000
Overhead	3,400
Other	2,000
Total	$ 45,000

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available: Staff - Dir. of Research, Pathologist, Secretary, Technician, and Laboratory Assistant.

Facilities - New lab. and new equipment, facilities for animals.

Available equipment: refrigerator, freezer, freeze-drying apparatus, Kymographs, waterbaths, analytical balance, pH meter autoclave, spectronic 20 colorimeter, homogenizer, demineralizer, etc.

Hospital of 400 beds with rich clinical material.

9. Additional Requirements:

(a) Personnel - 2 senior technicians with M.S. degrees $4,000 per year each.

(b) Equipment - 1. Spinco ultracentrifuge model L $6,000
2. Spinco electrophoresis $1600

10. Additional Information (Including relation of work to other projects and other sources of supply):

Other Projects

1. Detection of carcinoma antigen by use of the Schultz-Dale method in sera of patients with carcinoma. This project was supported by $6,000 for one year by U.S.P.H.S. Grant CS-9396 when director of project was elsewhere. A new application is being forwarded to U.S.P.H.S. for a study of this approach in carcinoma of the cervix.

2. Muhlenberg Hospital - a budget of $25,000 a year (excluding the pathologist) is set aside for research. Over the three year period requested for this project it would mean $75,000.

Signature /s/ Jack G. Makari, M.D.
Director of Project

Business Officer of the Institution Director

100354440

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

June 12 1956

From the desk of:

Reavis C. Sproull

To:-

1. If tumor formation is related to sex, the artificial conditions of tests may modify the sex behaviour so as to throw off the data accordingly.

2. Could it volume be determined more simply than by reconstructing models as done in the Temple study.

1003541441

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant /RENEWAL

Date: June 21, 1956

1. Name of Investigator: Dr. David E. Mann, Jr. and associates

2. Title: Associate Professor of Pharmacology

3. Institution & Address: Temple University School of Pharmacy
3223 North Broad Street
Philadelphia 40, Pennsylvania

4. Project or Subject: Effect of Tobacco Smoke and Tobacco Residues on the M.C.D.t (minimal carcinogenic dose, topically) of Methylcholanthrene in Mice.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Object: To determine the minimal carcinogenic dose (topically) of methylcholanthrene when applied biweekly to the interscapular space of mice; to confirm the inhibition produced by the prior administration of tobacco residue by observing its effect on skin carcinogenesis produced by the minimal carcinogenic dose of methylcholanthrene; to observe the effect of tobacco smoke on the M.C.D.t of methylcholanthrene; and, lastly, to determine whether or not the lung tumors produced by the application of methylcholanthrene are metastatic by the injection of alixarin red into the skin cancer and the subsequent observation histologically of lung tissue.

Procedure:

Part I: Determination of the M.C.D.t of Methylcholanthrene.

120 Carworth Farms mice of mixed sex will be divided into 12 groups of ten mice each. Each group of animals will receive twice a week a different concentration of methylcholanthrene in acetone ranging from 0.04% to 0.15%. Applications will continue for approximately 12 weeks and tumor development will be noted. From these data the M.C.D. topically can be ascertained.

1003541442

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Procedure:

Part II: Effect of Tobacco Residue on the M.C.D.$_t$ of Methylcholanthrene.

Thirty mice of mixed sex will receive biweekly applications of tobacco residue and methylcholanthrene (M.C.D.t) for approximately 12 weeks. Skin carcinogenesis will be noted. If tobacco residue inhibits methylcholanthrene-induced skin carcinogenesis no tumors should develop during this period.

Part III: Effect of Tobacco Smoke on the M.C.D.$_t$ of Methylcholanthrene.

Thirty mice of mixed sex will inhale tobacco smoke for a short period each day and will also receive the M.C.D.$_t$ of Methylcholanthren biweekly. Skin carcinogenesis will be noted during the 12 week period.

Controls: Fifteen mice will serve as controls and will remain untreated throughout the experiment.

Tobacco smoke alone:

Fifteen mice will inhale tobacco smoke for a short period each day but will not receive methylcholanthrene.

Part IV: Determination of Origin of Lung Cancer.

Thirty mice of mixed sex will receive one drop of 0.15% methylcholanthrene twice a week. As soon as skin cancers appear, each cancer will be injected with alizarin red at frequent intervals. After 20 weeks, all animals will be sacrificed and lungs will be examined for tumors. If lung tumors contain the dye, evidence of metastases will be indicated.

Materials and Methods:

Twelve-week old Carworth Farms mice (CFI) strain of mixed sex will be used throughout these studies. Each experiment will contain equal numbers of males and females which will be kept in individual cages.

The duration of each experiment is tentative and dependent upon the response of the animals to treatment. Histological studies will be made of the lungs and skin cancers of each animal (120).

10035441443

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan: TOBACCO INDUSTRY
150 EAST FORTY SECOND STREET

Salaries	3,000.00
Expendable Supplies	500.00
Permanent Equipment	500.00
Overhead	1,500.00 *
Other	
Total	5,500.00

Application (37.5%)

* At 15% $600.00
Total $4600.00

At 25% 900.00
Total $4900.00 P.C.H.

7. Anticipated Duration of Work:

32 weeks

8. Facilities and Staff Available:

Large animal house, adequate cages and research facilities.

STAFF: Department of Pharmacology
Dr. David E. Mann, Jr. Assoc. Prof.
One Graduate Assistant
One Instructor in Pharmacology
Department of Pathology
Dr. Frederic James (Prof. of Pathology)

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

5. Detailed Plan of Procedure (Use reverse side or additional space if necessary):

Signature
Director of Project
/s/ David E. Mann, Jr.

Business Officer of the Institution

1003511444

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TIRC Grant #8R1

Progress Report #2

Dr. David E. Mann, Jr.
Mr. Joseph Shanfeld
Temple University School of Pharmacy

May 1956

EFFECT OF TOBACCO SMOKE AND TOBACCO RESIDUES ON METHYLCHOLANTHRENE-INDUCED SKIN CARCINOGENESIS IN MICE

1003541445

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#8R1

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10035411446

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

RESULTS

TUMOR DEVELOPMENT

At the conclusion of the experiment on November 22nd, 1955, all pertinent data that had been obtained from the weekly record sheets of each animal (refer to accompanying sheet) were assembled and recorded on four large charts (one chart designated for each group), thus presenting in chronological order the progressive changes which developed with respect to the number and size of growths for each animal. From these data, five charts were subsequently constructed and entitled as follows:-

Chart 1 - NUMBER OF TUMORS IN EACH GROUP (Group A)

Chart 2 - NUMBER OF TUMORS IN EACH GROUP (Group B)

Chart 3 - NUMBER OF TUMORS IN EACH GROUP (Group C)

Chart 4 - NUMBER OF TUMORS IN EACH GROUP (Group D)

Chart 5 - NUMBER OF TUMORS IN ALL GROUPS (TOTAL AND AVERAGE)

NOTE:- Only those growths with at least one dimension (width vs. height) of one millimeter or greater were recorded on these charts. Tumors with both dimensions less than one millimeter were extremely difficult to identify correctly.

Graphs, in which the time (in weeks) was plotted against the number of tumors, were reconstructed from these charts and were entitled as follows:-

Graph 1 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group A)

Graph 2 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group B)

Graph 3 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group C)

Graph 4 - AVERAGE NUMBER OF TUMORS PER MOUSE (Group D)

Graph 5 - AVERAGE NUMBER OF TUMORS PER MOUSE (all groups)
Graph 6 - TOTAL NUMBER OF TUMORS (all groups)
Graph 7 - Mortality Rate in All Groups

Graphs 1 to 4 show curves for the male and female members of each

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group separately in order to demonstrate the sexual variation. The blue line represents the males and the red line the females. The black line represents the total (the sum of the male and female tumors) number of tumors per mouse in that particular group. Graph 5 gives the total number of tumors per mouse (regardless of sex) for all groups and provides a comparison of the groups on the same graph. Graph 6 gives the total number of tumors per group (irrespective of the number of survivors in each group) and shows curves very similar to Graph 5 except in the latter weeks of the experiment. Graph 7 shows the decided increase in mortality during the last few weeks of the experiment. A color key is included in all graphs to identify the various groups.

Number of Tumors in Each Group; Group A, Graph 1:-

Composite results regardless of sex:-

Groups A showed a rapid development in the number of growths commencing on the 10th week and reaching a plateau between the 14th and 15th weeks, and a peak on the 17th week. A sharp decline then occurred to the 18th week, whereupon the number of growths increased again, reaching a second plateau between the 19th and 20th weeks. This was followed by a decline (perhaps due to increased mortality in the latter weeks of the experiment).

Sexual Variations:-

The number of growths induced by the application of methylcholanthrene to the interscapular space of female mice were slightly higher than those recorded for males until the 21st week when the curve for females dropped below the male average.

Number of Tumors in Each Group; Group B, Graph 2:-

Composite results regardless of sex:-

Group B showed a rapid development starting on the 9th week and reaching the first peak on the 14th week. Then a sharp decline occurred to the 15th week, whereupon the curve rose again, reaching the highest peak on the 17th week. The curve declined gradually to the 22nd week, rising only

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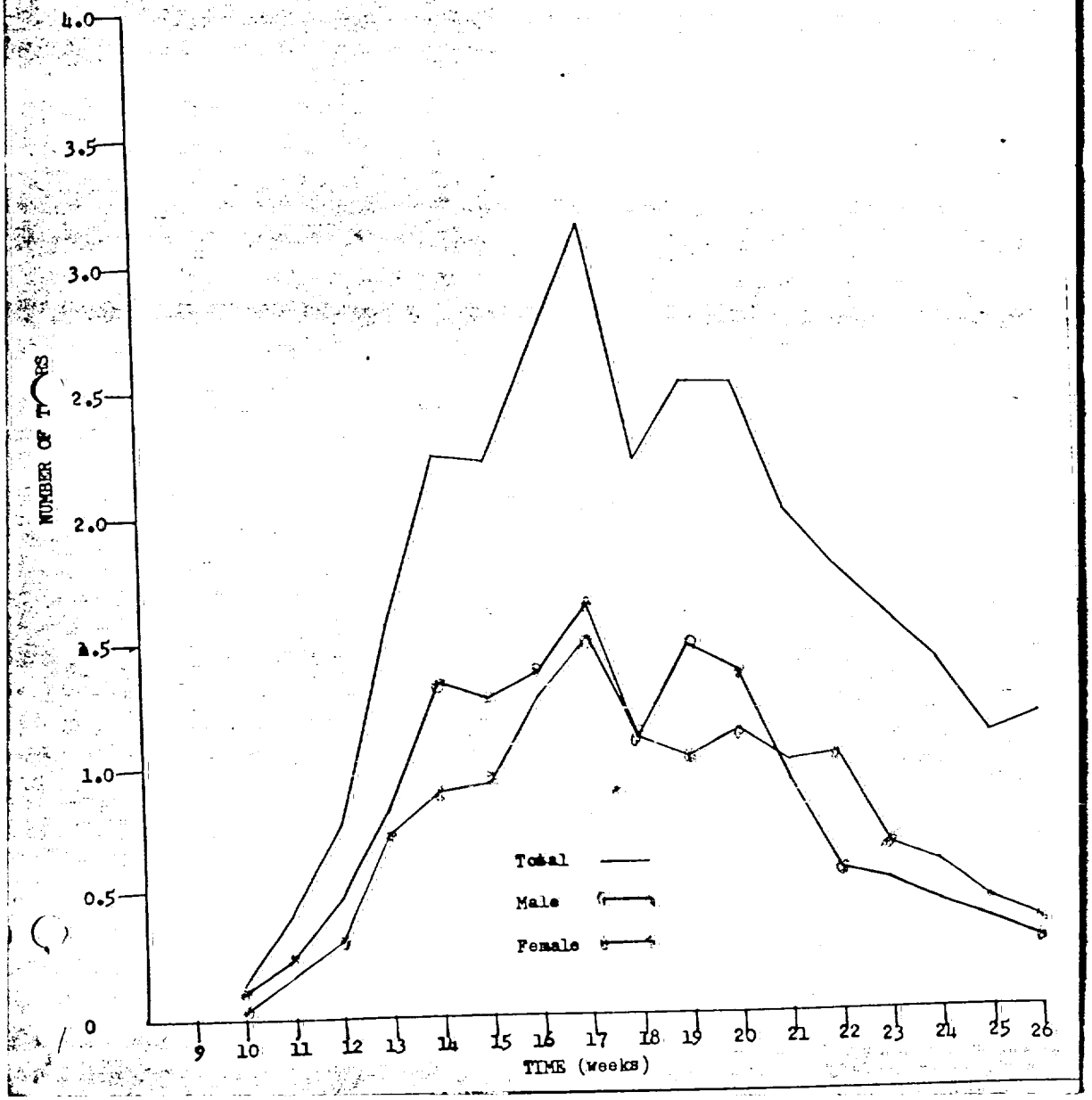
AVERAGE NUMBER OF TUMORS PER MOUSE (Group A)

Graph 1



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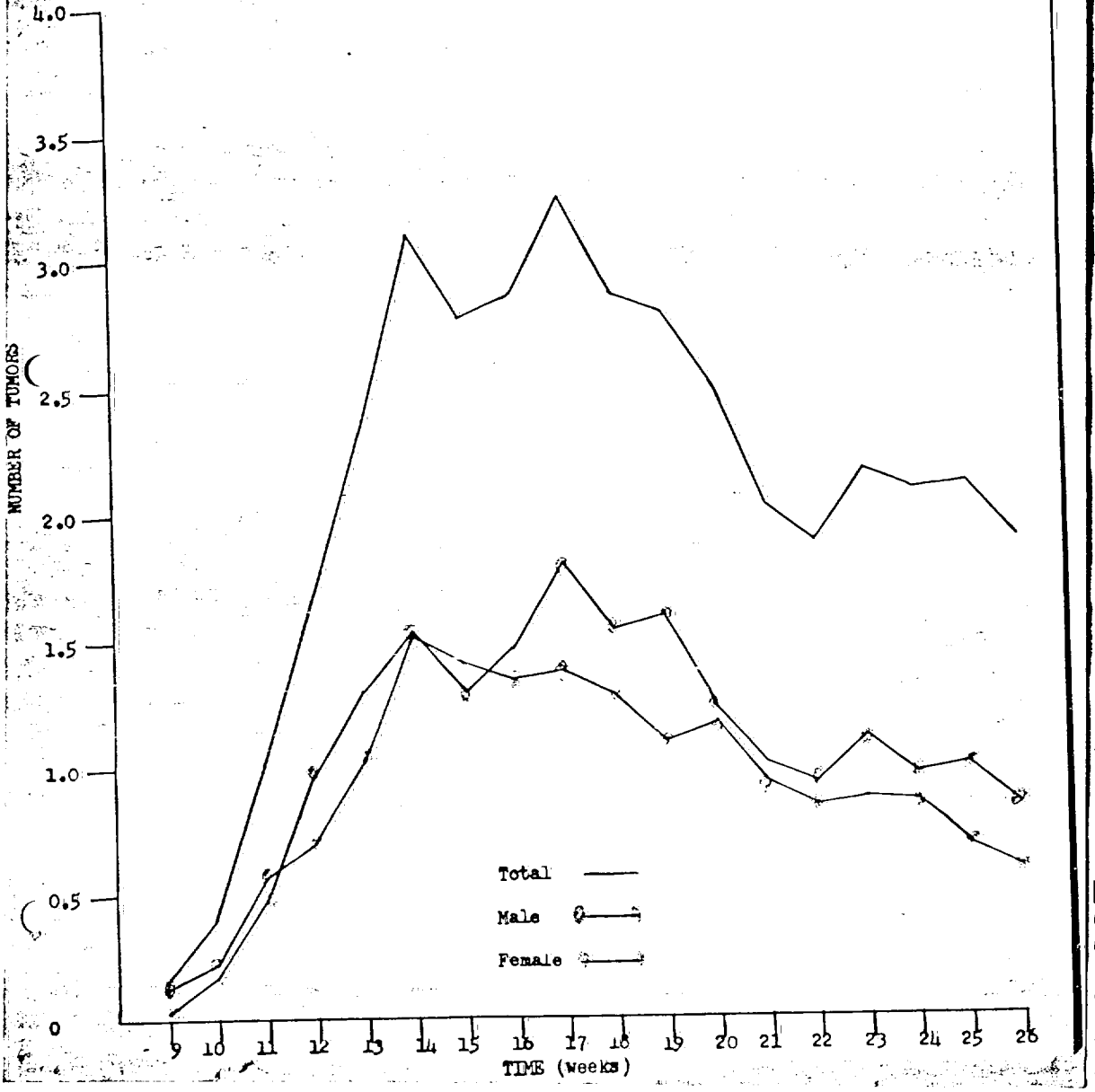
AVERAGE NUMBER OF TUMORS PER MOUSE (Group B)

Graph 2



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slightly to the 23rd week. A plateau then developed to the 25th week, followed by a decline. Group B had a slightly greater number of tumors than A.

Sexual Variations:-

Both sexes showed a similar pattern at the onset. However, after the 15th week, females showed a sharper increase in number of growths than the males.

Number of Tumors in Each Group; Group C, Graph 3:-

Composite results regardless of sex:-

Initial growths occurred during the 9th week of the experiment, whereupon a sharp increase developed reaching the first peak by the 14th week. A decline then occurred to the 15th week followed by a gradual increase to the 18th week. A sharp increase then developed which reached the highest peak on the 20th week. This was followed by a sharp decline to the 22nd week; an increase to the 24th week; leveling off on the 25th week, and finally a rapid decline.

Sexual Variations:-

Both curves were similar until the 13th week. Males then showed a greater number of tumors than females to the 23rd week.

Number of Tumors in Each Group; Group D, Graph 4:-

Composite results regardless of sex:-

Growths began on the 9th week of the experiment. A rapid increase developed shortly after the onset to the 16th week, leveling off to the 19th week. A sharp increase then occurred (slightly higher than for Group C) which attained the highest peak on the 20th week. A decline (resembling that of Group C) then was noted to the 22nd week.

Sexual Variations:-

The curves were similar for each sex to the 11th week when males revealed a much greater number of tumors than females to the 25th week.

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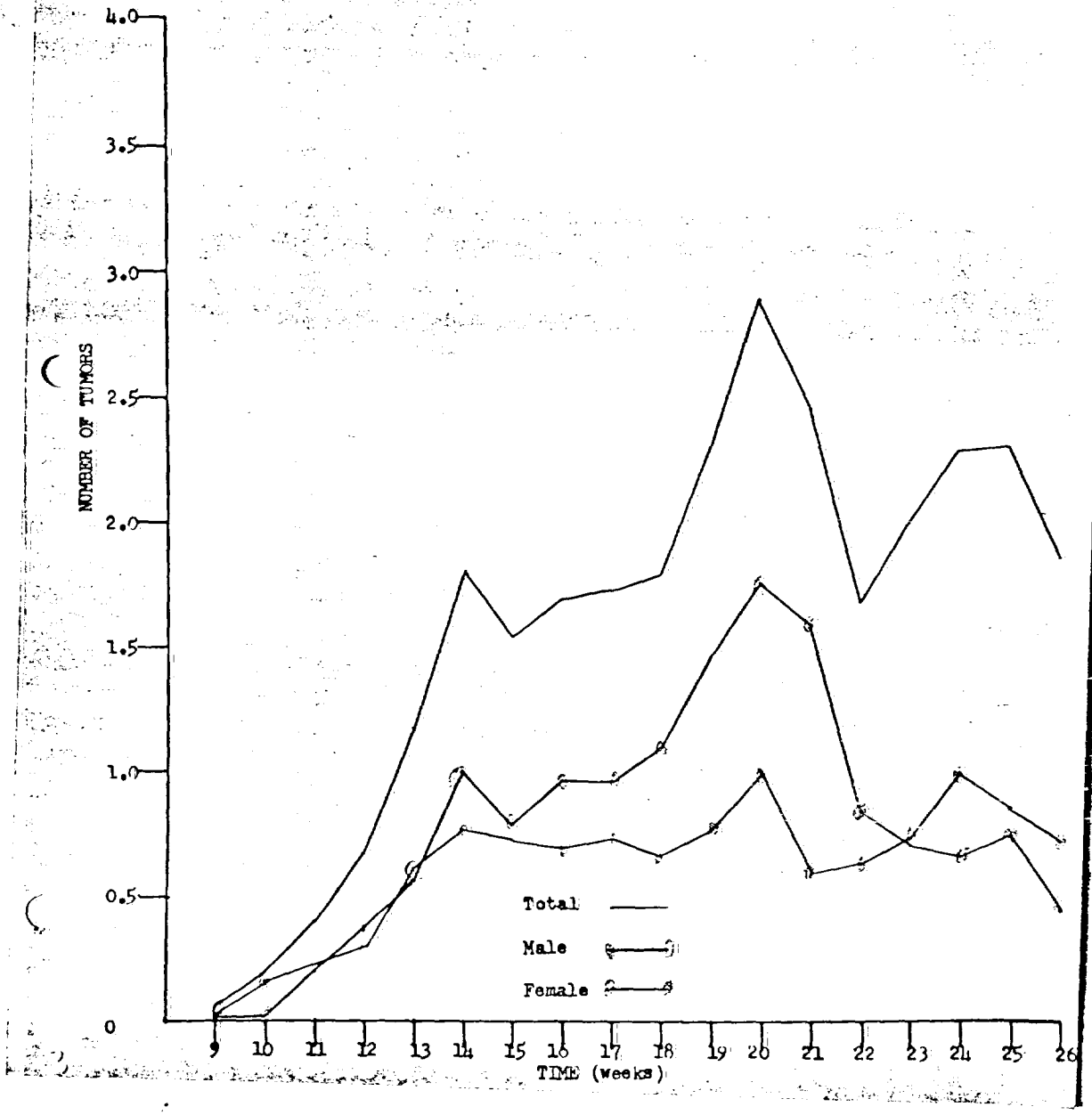
AVERAGE NUMBER OF TUMORS PER MOUSE (Group C)

Graph 3



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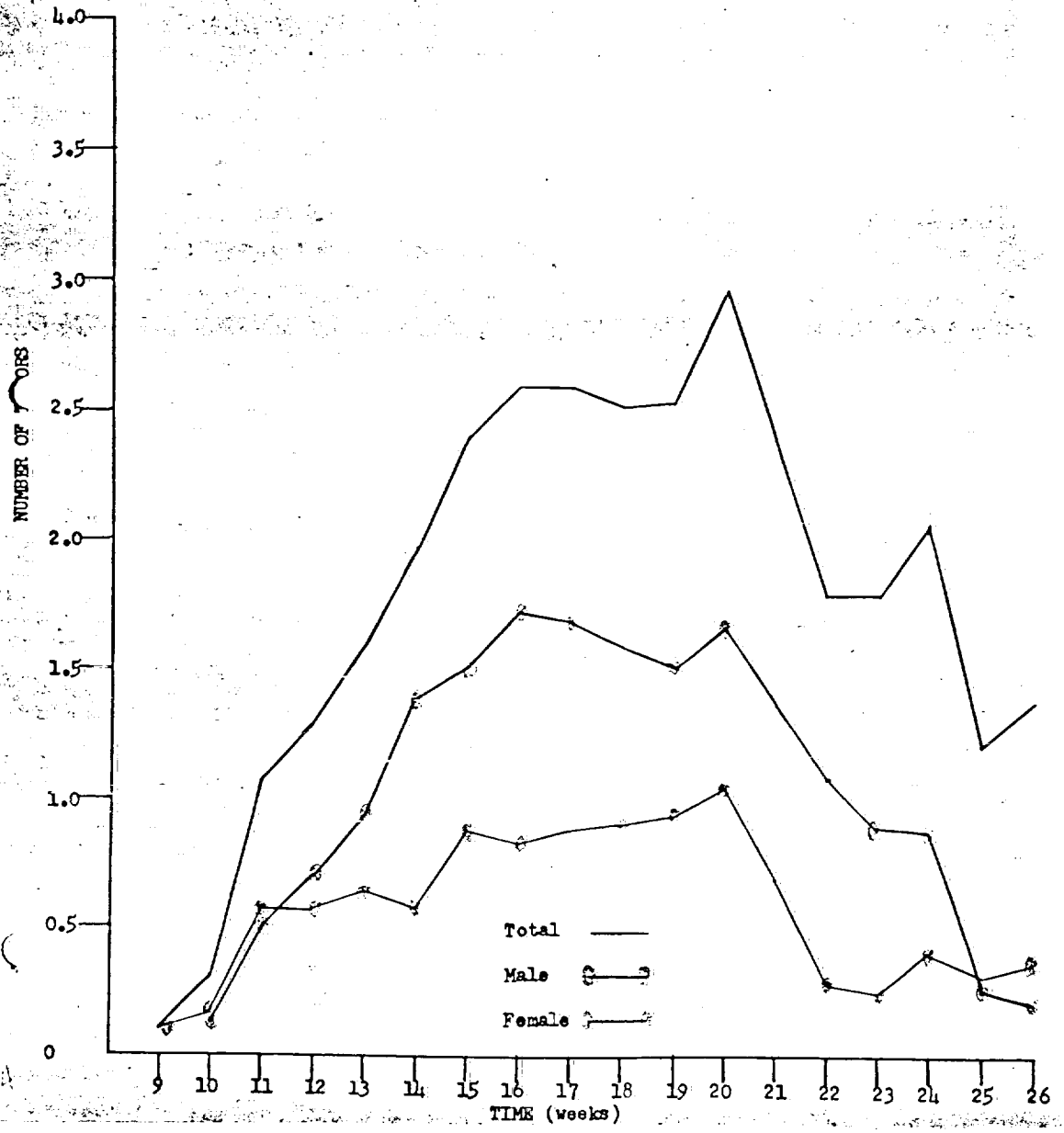
AVERAGE NUMBER OF TUMORS PER MOUSE (Group D)

Graph 4



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SUMMARY

According to Graph 5, Group C (tobacco residue and methylcholanthrene applications) showed a well-defined inhibition in the development of the number of tumors from the 14th week to the 18th week, and a slower onset of tumor formation than its control group Group D (acetone and methylcholanthrene applications). Group C showed fewer number of tumors than all of the other groups from the 11th week to the 19th week. Groups A, B, and C showed three distinct peaks. These peaks were followed by a decrease or a static condition with respect to the number of growths. This may be due to the superimposition of some defensive response mechanism of the animal to the carcinogen. Group D developed a plateau in place of the initial peak observed in the other groups. During this period the number of growths in the control Group D was appreciably greater than those in Group C. The second peak in Groups A and B occurred three weeks before the second peak of Group C. The third peak of all groups occurred during the latter part of the experiment where the increase in mortality makes the interpretation difficult.

According to the data collected, Group C showed a delay in the onset of tumor formation as compared to its control and had a fewer number of growths than all of the other groups during the period of the experiment when most animals were still alive. It is difficult to draw accurate conclusions from data obtained during the final weeks of the experiment because of the higher mortality rate.

From these data it appears that the biweekly application of tobacco residue definitely inhibits the number of tumors in Group C for a significant period of time, inferring that tobacco residue contains a weak carcinogen with a chemical configuration similar to that of methylcholanthrene.

Group B (tobacco smoke and methylcholanthrene) showed the largest number of tumors than any other group until just after the 19th week. It may

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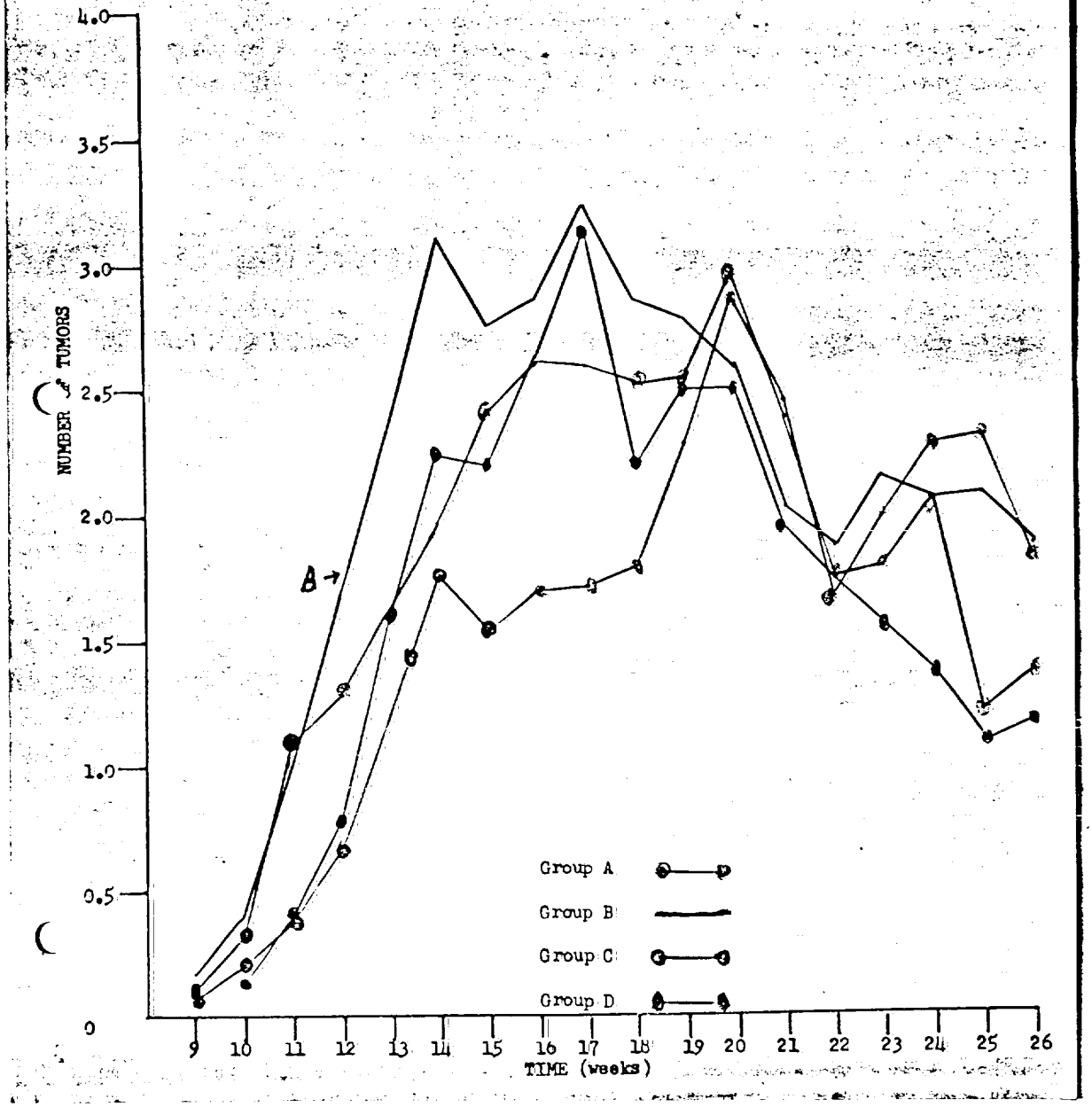
AVERAGE NUMBER OF TUMORS PER MOUSE (all groups)

Graph 5



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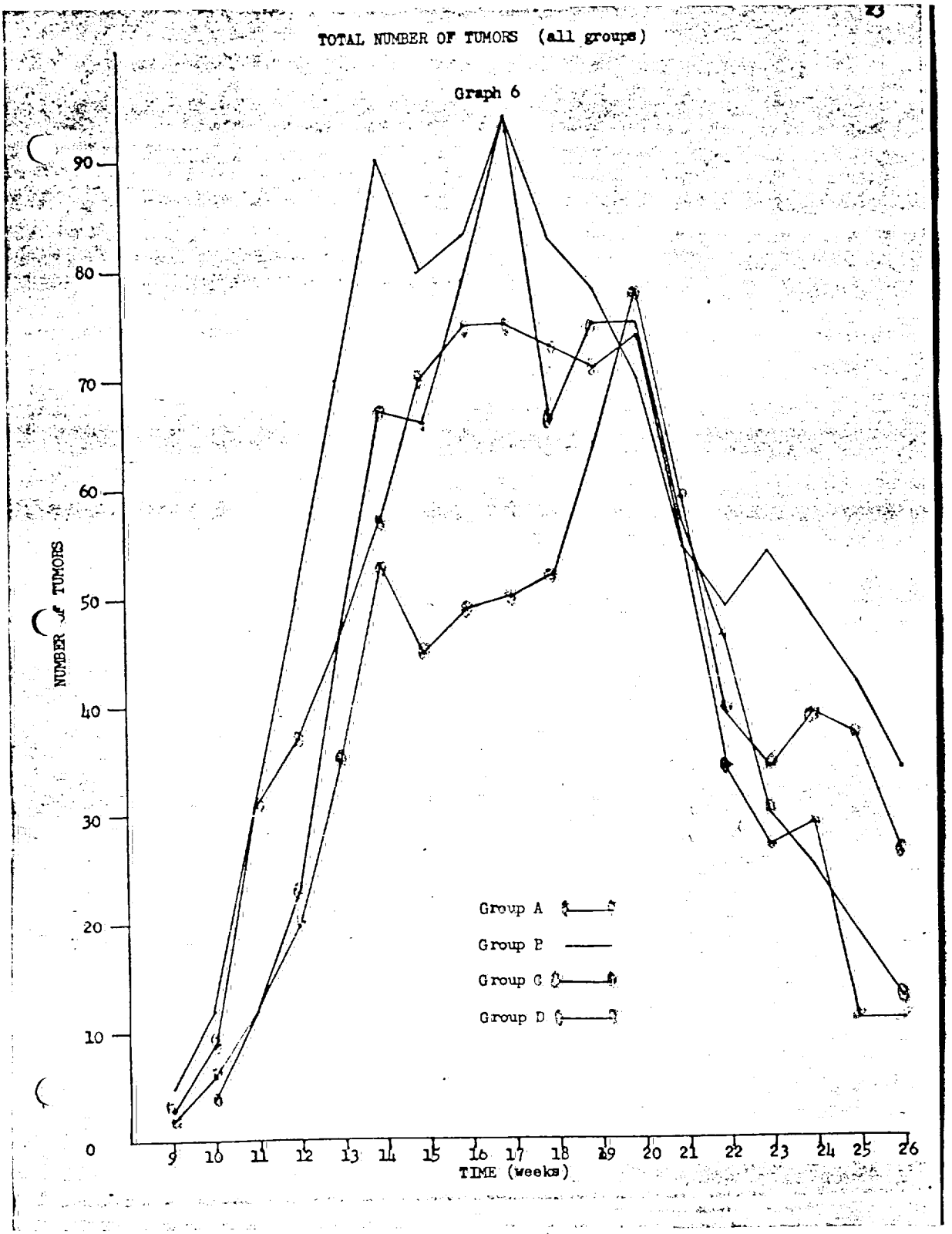
TOTAL NUMBER OF TUMORS (all groups)

Graph 6



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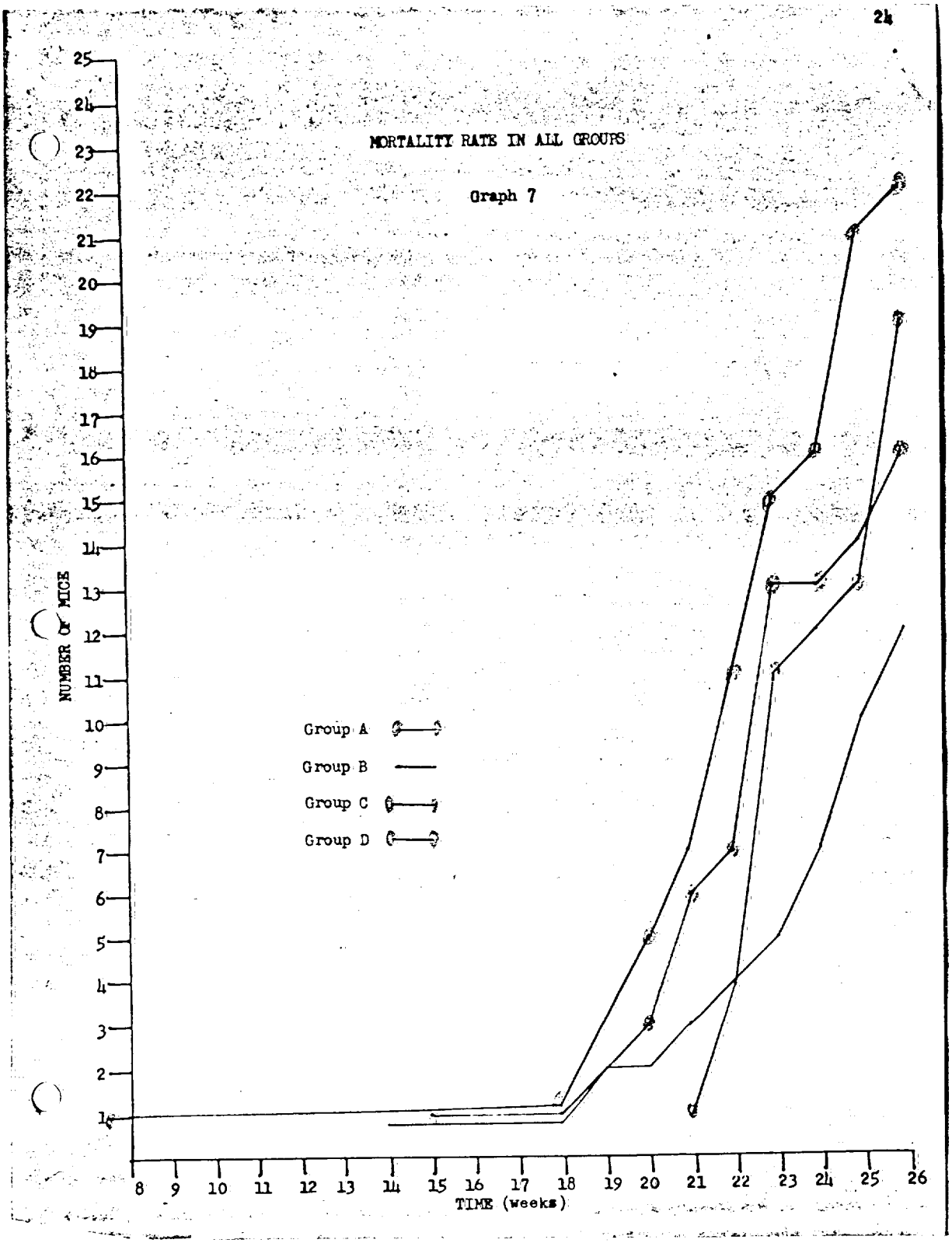

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be noted that Group B had the lowest mortality after the 22nd week of the experiment.

A more definite conclusion may be drawn after tumor volume has been determined for each animal. This requires a great deal of time as many of the tumors observed were irregular in form. In order to obtain the volume of each tumor of irregular shape it is necessary to reconstruct the growth in clay or similar modeling material from the dimensions and sketches previously taken every week for all animals. This reconstructed tumor in clay or other suitable material can then be weighed and from the density of the material the volume calculated. This necessitates the construction of several hundred models to obtain data of tumor size.

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TIRC Grant #8R1
David E. Mann, Jr., M.D.
Temple University

Progress Report #3
June 20, 1956

Pathology Report

Effects of Tobacco Smoke and Tobacco Residue on Methylcholanthrene-Induced Skin Carcinogenesis in Mice

By: Dr. Frederic James, Temple University School of Dentistry, Philadelphia, Pa.

In compiling a pathological report on this research project suffice it to say that a preliminary examination was undertaken microscopically on all tumor sections regardless of the group in which the mouse was classified. This was done to determine variations, if any, as to the type of tumor. As it happened all growths, regardless of their size or location showed very little departure from a generalized pattern. The same held true during the period the animals were living. In this instance the only variations seen were those of shape, size and consistency as is well demonstrated by the selected series of Kodachrome prints. It was very interesting to note as growth continued that some assumed a raised circular characteristic, while others showed variable horn-like formations. In most instances the growths were loosely attached to the deeper structures and when removed for fixation often fell apart due to central caseation, although on the surface most of them were hard as evidence of hyperkeratosis. Once a growth was removed from its attachment to the thoracic wall, which was the case in most instances, the thoracic organs were free within their cavity. This seemed quite remarkable in view of the extensive nature of so many of the growths. In many cases the head of the animal was either drawn to the right or left side or held positionally central when the growth almost ringed the neck. Invariably the ear and forelimb or ears and forelimbs were contained in the mass, but regardless of all these variations in a forward, lateral or backward direction the growths appeared to be quite superficial. It was remarkable to note that only in a few instances at the time of autopsy was there any evidence of secondary (metastatic) growths in lung, liver, kidney or pancreas. Even in cases where nodules were suspected the matter was only given tentative consideration pending microscopic examination.

Each section examined microscopically showed the following characteristics to a greater or lesser degree, depending upon the size of the neoplasm and the time of death, whether occasioned by the growth or induced at the end of the experimental period:-

1. A progressive increase in the thickness of the stratum corneum which in practically every instance presented an extreme hyperkeratosis.

2. Broadening, with resultant fusion of the interpapillary processes in addition to an extreme depth of penetration into the submucosa.

3. The cells of the invading epithelium varied considerably in-so-far-as:-

   (a) The outermost layer resembled the basal layer of the epithelium

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(b) The innermost layers lacked, in most instances, a clear differentiation. Some of the cells were round, others elongated with variations in their cytoplasmic staining.

(c) Many of the nuclei of the cells exhibit hyperchromatism.

(d) Mitoses were numerous and for the most part atypical.

4. "Cell nests" or squames are abundant and in the superficial layers of the neoplasm are represented as keratinized cores devoid of any cellular elements.

5. Squames in the deeper regions show more definition since they are composed of several layers of flattened "prickle-cells" concentrically arranged about a core of homogenous acidophile hyaline.

6. Many of the "cell nests" by virtue of their breakdown consist of fragments of keratinous material, as well as granular and necrotic debris.

7. Numerous polymorphonuclear leucocytes infiltrate into some of the more recently formed "cell nests" although the presence of these cells is not limited since they are seen in greater numbers scattered among the epithelial cells and the connective tissue elements.

8. Between the epithelial processes the connective tissue varies. In some areas it is loose in texture, in others it is definitely fibrous in character.

9. In the tissue spaces many epithelial cells have become detached, either singly or in clusters and are intermingled with the inflammatory cells which are composed for the most part of polymorphonuclear leucocytes along with lymphocytes, plasma cells and histiocytes.

10. Deposits of pigment, hemosiderin, are quite abundant.

11. Necrotic tissue that presented, prior to sectioning, a soft dryish, crumbly, cheesy mass due mainly to fatty degenerative changes. This sphacelus in some areas is devoid of any structural characteristics, in others it is admixed with degenerated cellular elements of both tissue and blood (leucocytes).

12. The predominance of polymorphonuclear leucocytes presented a most interesting microscopic picture in most instances.

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In summarizing this series of microscopic examinations one can only repeat that in spite of the grouping of the animals and the methods employed in applying the methylcholanthrene the neoplasias per se followed a pattern of similarity in that structural form and appearances while the animals lived did not produce any difficulties in diagnosis in that squamous-cell carcinoma was the rule. It can also be confirmed that in those cases that manifested true metastases in the lungs, the diagnosis was the same.

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Source: https://www.industrydocuments.ucsf.edu/docs/fvm0000

ANALYSIS OF SECTIONS OF LUNG

GROUP "A"

No. 1. Apparently little change from the normal pattern.

No. 2. Interstitial pneumonitis with some bronchiolitis.

No. 3. Interstitial exudate and interstitial cellular infiltration. Hyalinization with eosinophilic staining. Degenerated polymorphonuclear leucocytes.

No. 4. Primarily a pneumonitis.

No. 5. Interstitial broncho-pneumonia.

No. 6. Broncho-pneumonia. Alveolar obliteration. Extreme cellular infiltration. Consolidation in many areas.

No. 7. Apparently little change from the normal pattern.

No. 8. Acute pneumonitis and bronchiolitis.

No. 9. Acute pneumonitis and bronchiolitis.

No. 10. Apparently little change from the normal pattern.

No. 11. Apparently little change from the normal pattern.

No. 12. Acute pneumonitis and bronchiolitis. Hyalinization with some consolidation.

No. 13. Slight pneumonitis.

No. 14. Acute pneumonitis with marked cellular infiltration.

No. 15. Slight pneumonitis.

No. 16. EXTREME METASTASIS COMPARABLE TO PRIMARY TUMOR ON THE SKIN. GREATER PART OF LUNG INVOLVED. KERATINIZED "CELL NESTS" ABUNDANT AND PATTERN TYPICAL.

No. 17. Apparently little change from the normal pattern.

No. 18. Acute pneumonitis and bronchiolitis.

No. 19. Slight pneumonitis and bronchiolitis.

No. 20. Slight pneumonitis.

No. 21. Pneumonitis.

No. 22. Apparently little change from the normal pattern.

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No. 23. MARKED METASTASIS IN THE LUNG TISSUE AND ABOUT THE BRONCHI AND BRONCHIOLES. DEGENERATION IN CENTRAL PORTION OF TUMOR. "KERATINIZED CELL NESTS" PRESENT BUT SMALL TO MEDIUM IN SIZE.

No. 24. Acute pneumonitis and bronchiolitis. Much evidence of hemosiderin.

No. 25. METASTASIS COMPARABLE TO PRIMARY TUMOR ON THE SKIN. KERATINIZED "CELL NESTS" WELL DEVELOPED AS WELL AS OTHER MALIGNANT CHARACTERISTICS OF THE CELLULAR ELEMENTS - ANASPLASIA, PLEOMORPHISM AND HYPERCHROMATISM.

No. 26. Broncho-pneumonia. Excessive exudate. Cellular infiltration. Areas of emphysema.

No. 27. Acute pneumonitis and bronchiolitis.

No. 28. Broncho-pneumonia with marked exudate and cellular infiltration.

No. 29. Apparently little change from the normal pattern.

No. 30. Pneumonitis and bronchiolitis.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "A" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION THAT:

No. 16

No. 23 ALONE IN THE SERIES SHOWED METASTATIC DEPOSITS IN THE LUNG.

No. 25

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

ANALYSIS OF SECTIONS OF LUNG

GROUP "B"

No. 31. Apparently little change from the normal pattern.

No. 32. Pneumonitis and cavitation of lung tissue. Interstitial cellular infiltration.

No. 33. Acute pneumonitis and extreme bronchiolitis. Congestion very marked.

No. 34. Apparently little change from the normal pattern.

No. 35. EXTREME METASTASES COMPARABLE TO PRIMARY TUMOR ON SKIN. KERATINIZED "CELL NESTS" WELL DEFINED IN ADDITION TO OTHER MALIGNANT CHARACTERISTICS.

No. 36. Pneumonitis with apparently some cavitation of lung tissue.

No. 37. Apparently little change from the normal pattern.

No. 38. Apparently little change from the normal pattern.

No. 39. EXTREME METASTASIS ALMOST OBLITERATING LUNG TISSUE. LARGE NUMBER OF KERATINIZED "CELL NESTS" SHOWING CENTRAL NECROSIS.

No. 40. Pneumonitis.

No. 41. Pneumonitis and bronchiolitis. Abundant deposits of hemosiderin.

No. 42. Acute broncho-pneumonia. Exudate excessive. Marked interstitial cellular infiltration. Alveoli distended and many show tissue breakdown.

No. 43. Pneumonitis.

No. 44. A SECTION OF THE LUNG TISSUE SHOWS A SMALL AREA OF METASTASIS. KERATINIZED "CELL NESTS" ARE PRESENT BUT ARE VERY IMMATURE.

No. 45. Pneumonitis and bronchiolitis.

No. 46. SMALL AREA OF METASTASIS EXTENDING INTO LUNG TISSUE FROM THE SURFACE OF LOBE. COMPOSED MAINLY OF MALIGNANT CELLS. NO KERATINIZED "CELL NESTS."

No. 47. Apparently little change from the normal pattern.

No. 48. DEFINITE METASTASIS COMPARABLE TO PRIMARY TUMOR ON SKIN. KERATINIZED "CELL NESTS" AND OTHER MALIGNANT CHARACTERISTICS.

No. 49. Acute pneumonitis and bronchiolitis.

No. 50. Apparently little change from the normal pattern.

No. 51. Small patches of evident pneumonitis with some emphysematous changes.

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No. 52. EXTREME METASTASES COMPARABLE TO PRIMARY TUMOR ON SKIN. KERATINIZED "CELL NESTS" IN ADDITION TO BEING LARGE ARE ALSO NUMEROUS AND REVEAL VARIOUS PATTERNS. TWO METASTATIC AREAS ARE DEMONSTRABLE. MALIGNANT CELLS ARE ANASPLASTIC, PLEOMORPHIC AND SHOW MARKED HYPERCHROMATISM.

No. 53. Apparently little change from the normal pattern.

No. 54. Pneumonitis and bronchiolitis.

No. 55. Slight pneumonitis. Lung tissue shows little involvement.

No. 56. Pneumonitis. Lung tissue shows considerable vascular congestion.

No. 57. MARKED METASTASIS WITH VARIABLE SIZE KERATINIZED "CELL NESTS" AND OTHER MALIGNANT CHARACTERISTICS. THE TUMOR IS COMPARABLE IN GENERAL DESIGN TO THE PRIMARY TUMOR ON THE SKIN.

No. 58. Slight pneumonitis. Changes not too well defined.

No. 59. Pneumonitis and bronchiolitis. Extensive cellular infiltration.

No. 60. Pneumonitis and bronchiolitis with extreme edema and congestion.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "B" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION THAT:

No. 35
No. 39
No. 44
No. 46 ALONE IN THE SERIES SHOWED METASTASES IN THE LUNG
No. 48
No. 52
No. 57

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ANALYSIS OF SECTIONS OF LUNG

GROUP "C"

No. 61. Pneumonitis and bronchiolitis. Alveolar congestion marked.

No. 62. No lung on slide.

No. 63. Pneumonitis and bronchiolitis. Marked arterial engorgement.

No. 64. Mainly a bronchiolitis. Slight involvement of the alveoli.

No. 65. Pneumonitis and bronchiolitis. Marked arterial congestion.

No. 66. Apparently little change from the normal pattern.

No. 67. Diffuse pneumonitis with patches of broncho-pneumonia. Degeneration of the alveoli many of which are almost obliterated. Congestion extensive and solid both in the lung tissue itself and about the bronchioles.

No. 68. Pneumonitis.

No. 69. Acute extensive pneumonitis with marked alveolar distension. Cellular infiltration extreme. Exudate pronounced. Hyalinization marked.

No. 70. Hemorrhagic interstitial pneumonitis and bronchiolitis.

No. 71. Slight pneumonitis and bronchiolitis.

No. 72. Lung almost normal although slight cellular infiltration is seen in some areas.

No. 73. Apparently little change from the normal pattern.

No. 74. Mainly interstitial pneumonitis.

No. 75. METASTASES AND LUNG ABSCESS. KERATINIZED "CELL NESTS" NUMEROUS ALONG WITH CLUSTERS OF MALIGNANT CELLS. BREAKDOWN OF LUNG TISSUE VERY PRONOUNCED. SOLIDARITY OF TISSUE IN TUMOR AREA.

No. 76. Acute pneumonitis with marked alveoli distension. Cellular infiltration extensive. Exudate pronounced.

No. 77. Lung appears quite emphysematous and well-infiltrated with polymorphonuclear leucocytes.

No. 78. Pneumonitis and bronchiolitis. Much hemorrhagic congestion.

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GROUP "C" - Cont'd.

No. 79. Interstitial pneumonitis.

No. 80. Pneumonitis with marked edema about the alveoli.

No. 81. Pneumonitis with much fibrinous exudate. Alveoli distended.

No. 82. No lung on slide.

No. 83. Apparently little change from the normal pattern.

No. 84. Pneumonitis and bronchiolitis. Marked hemorrhagic congestion.

No. 85. Very mild cellular infiltration of tissue.

No. 86. Pneumonitis and bronchiolitis with many areas of emphysema. Much hemosiderin.

No. 87. Pneumonitis with fibrinous exudate. Marked cellular infiltration. Extensive hemorrhages. Some areas of emphysema.

No. 88. Pneumonitis with much cellular infiltration and exudate.

No. 89. Pneumonitis with bronchiolitis.

No. 90. Broncho-pneumonia with extreme solidarity of lung tissue. Cellular infiltration extensive. Obliteration of bronchioles and alveoli.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "C" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION:

No. 75. ALONE IN THE SERIES SHOWED METASTATIC DEPOSITS IN THE LUNG.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

ANALYSIS OF SECTIONS OF LUNG

GROUP "D"

No. 91. Pneumonitis and bronchiolitis. Extensive cellular infiltration, edema, and emphysema.

No. 92. Slight pneumonitis. Some cellular infiltration. Hemosiderin extreme.

No. 93. Acute pneumonitis with marked alveolar distension. Cellular infiltration extreme. Exudate pronounced. Areas of hyalinization.

No. 94. Apparently little change from the normal pattern.

No. 95. Apparently little change from the normal pattern.

No. 96. METASTATIC DEPOSITS IN LUNG ARE PRESENT BUT LIMITED IN LOCATION TO A SMALL AREA OF THE TISSUE. MAINLY COMPOSED OF MALIGNANT CELLS. NO EVIDENCE OF ANY KERATINIZED "CELL NESTS."

No. 97. Broncho-pneumonia. Alveoli considerably broken down. Little intra-alveolar exudate. Interstitial cellular infiltration.

No. 98. Pneumonitis and bronchiolitis. Some emphysema.

No. 99. Pneumonitis and bronchiolitis.

No. 100. Broncho-pneumonia. Solidarity of alveoli. Marked cellular infiltration and extensive emphysema.

No. 101. Pneumonitis showing hyalinization of alveoli at apex. Cellular infiltration.

No. 102. Acute pneumonitis. Exudate excessive. Marked interstitial cellular infiltration.

No. 103. Broncho-pneumonia. Extensive cellular infiltration. Some emphysema.

No. 104. Pneumonitis and bronchiolitis.

No. 105. Pneumonitis and bronchiolitis. Some areas of emphysema.

No. 106. Acute pneumonitis with extreme cellular infiltration. Exudate very pronounced. Lung markedly congested.

No. 107. Slight pneumonitis and bronchiolitis.

No. 108. Broncho-pneumonia. Solidarity of lung at apex very pronounced. Intercellular edema. Some emphysema.

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GROUP "D" - Cont'd.

No. 109. SMALL PATCH OF METASTASIS IN LUNG SUBSTANCE ABOUT A BRONCHIOLE. ALSO MASSES OF EXTENSIVE METASTASES COMPOSED MAINLY OF MALIGNANT CELLS. SOME EARLY KERATINIZATION. EVIDENCE OF BRONCHO-PNEUMONIA.

No. 110. SMALL PATCH OF METASTASIS COMPOSED MAINLY OF MALIGNANT CELLS. NO "CELL NESTS." THIS IS IN CENTRAL AREA OF TISSUE. ALSO AN AREA OF EXTENSIVE METASTASIS COMPOSED MOSTLY OF MALIGNANT CELLS, ALTHOUGH THERE IS SOME EARLY EVIDENCE OF KERATINIZATION IN THIS REGION.

No. 111. Marked hyalinization. Extreme interstitial exudate. Acidophilic staining marked.

No. 112. Pneumonitis. Cellular infiltration. Edema of alveoli.

No. 113. Pneumonitis and bronchiolitis.

No. 114. Pneumonitis and bronchiolitis.

No. 115. Interstitial broncho-pneumonia. Breakdown of alveoli. Little intra-alveolar exudate. Marked cellular infiltration. Many areas are solid with cells.

No. 116. MARKED METASTASIS WITH LARGE AREAS OF TISSUE DESTRUCTION. CELLULAR INFILTRATION EXTREME. EXUDATE MARKED.

No. 117. Slight pneumonitis.

No. 118. Slight cellular infiltration about vessels and bronchi.

No. 119. SMALL PATCH OF METASTASIS AROUND A BRONCHIOLE. SOME CELLULAR INFILTRATION IN OTHER AREAS.

No. 120. Broncho-pneumonia. Fibrinous exudate. Much deposit of hemosiderin.

NOTE:

THE ABOVE TABULATION REPRESENTS THE GENERAL ANALYSIS OF GROUP "D" SERIES AND REVEALS UPON MICROSCOPIC EXAMINATION THAT:

No. 96
No. 109
No. 110 ALONE IN THE SERIES SHOWED METASTATIC DEPOSITS
No. 116 IN THE LUNG
No. 119

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

SUMMARY BY GROUPS

GROUP "A" in which thirty (30) mice (controls) received METHYLCHOLANTHRENE twice a week.

RESULTS

No. 16 showed extreme metastases comparable to the growth characteristics as seen in the primary skin tumor. A greater portion of the lung tissue was involved. Keratinized "cell nests" were abundant and the general malignant pattern was typical.

No. 23 shows characteristics not as extreme as in No. 16 but more localized. In this case the "cell nests" are less keratinized and the lung tissue is indented from its surface inwards and there are definite necrotic changes. Other malignant characteristics are well defined.

No. 25 shows two well circumscribed areas of malignancy with very pronounced keratinized "cell nests" formations. These keratinized areas are well defined in their acidophilic staining qualities. The cells throughout the tumor mass are pleomorphic, anaplastic and reveal much hyperchromatism.

GROUP "B" in which thirty (30) mice inhaled tobacco smoke (in a closed smoking chamber) for a short period each day and also received METHYLCHOLANTHRENE twice a week.

RESULTS

No. 35 shows extreme metastases in the lung involving a large area of tissue. In this section the lung is solid with growth and the keratinized "cell nests" are particularly large and confluent from the margin to the central region of the tissue. Beyond this the keratinized areas are smaller and the malignant cells are closely packed together and reveal anaplastic, pleomorphic and hyperchromatic characteristics.

No. 39. shows a dense mass of neoplastic growth almost obliterating the lung tissue. In addition to the extreme number of keratinized "cell nests" many show a disintegrated central portion (necrosis). The squamous cell characteristics are very pronounced and the overall cellular features show this growth to be not only extensive but a rather rampant process. The cellular appearances are the same as described in the other neoplasias.

No. 44. shows a very small patch of malignancy close to the lung apex. The keratinized "cell nests" in comparison with other growth descriptions are very small; however, there is no doubt about the metastatic nature of the area.

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SUMMARY BY GROUPS - Cont'd.

No. 46 shows a metastatic area along the lung border of small dimensions but definite. This area is composed for the most part of malignant cells with similar characteristics referred to in the other metastatic growths with the one exception that there are no keratinized "cell nests" present.

No. 48 shows extreme metastases with similar characteristics as referred to in Section No. 35. The pattern and arrangement of the cells are the same and for that reason repetition is unnecessary.

No. 52. shows by far the largest area of involvement in this group. Even on macroscopic examination of the tissue two large masses are seen which upon microscopic examination show the same pattern of keratinized "cell nest" formations and the same striking effort at cell de-differentiation and keratinization. The anaplastic, pleomorphic and hyperchromatic characteristics are prominent among the malignant cells.

No. 57 shows extreme metastases in two areas, one of which is intimately related to a main bronchus. The squamous cell characteristics are well defined and again keratinized "cell nests" formations are present throughout the tumor. The malignant cells are closely packed in some areas and there is marked solidarity of the lung tissue. The same additional features mentioned in other growths are prominent in this section.

GROUP "C" in which thirty (30) mice were administered topically tobacco residue extract (acetone) and METHYLCHOLANTHRENE twice a week in that order.

RESULTS

No. 75 shows a large circumscribed mass of tumor with numerous small keratinized "cell nests" about a bronchus. The characteristics of the malignant cellular elements exhibit anaplasia, pleomorphism and hyperchromatism as is seen in other tumors of the series. Solid masses of inflammatory cells are present resulting in consolidation in some areas. Emphymatous changes are quite pronounced and in many regions the lung tissue shows considerable breakdown. A broncho-pneumonic pattern is quite evident.

GROUP "D" in which thirty (30) mice received topically acetone and METHYL-CHOLANTHRENE twice a week.

RESULTS

No. 96 shows a mass of malignant cells localized on the margin of the lung tissue. Although this area is small, many of the cells show a tendency to neoplastic characteristics. Numerous polymorphonuclear leucocytes are contained in the field. The change is not too striking from that of an inflammatory picture. Most of the lung tissue appears relatively normal except for local areas of cellular infiltration.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

SUMMARY BY GROUPS - Cont'd.

No. 109 shows a small patch of malignancy within the lung tissue about a bronchiole. The cells reveal definite malignant characteristics in shape, size and staining appearances.

No. 110 shows a large mass of growth that is quite circumscribed and related to the apex of the lung. The tumor is composed for the most part of malignant cells which have caused extreme bulging of the tissue. No keratinized areas are present although there are patches of acidophile hyaline. The cells are unquestionably neoplastic in nature.

No. 116 shows multiple metastatic growths in both portions of lung tissue. Some detached portions of growth are seen about a bronchus. There are many areas of keratinized "cell nests" and the general malignant characteristics of the cells is very striking - anaplasia, pleomorphism and marked hyperchromatism. Many of the cells appear as tumor giant cells.

No. 119 shows a small patch of metastatic growth about a bronchus. Except for this area all other portions of lung tissue appear relatively normal.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

DISCUSSION

In analyzing the groups together rather than individually it will be noticed that with few exceptions there was present in each mouse evidence of respiratory pathology. Not all animals used in the course of the research died by virtue of the appearance or size of the tumor. A considerable number were put to death by artificial means to culminate the work within the allotted period of time.

It was also very surprising to note that irrespective of the size of the primary tumors, the number of visible microscopic metastases in the lungs were relatively few and these varied both in size and appearances. Naturally one might ask the question as to what number of metastases might have developed had the animals, whose deaths were produced artifically, had been permitted to survive to a natural death, by virtue of the effects of the neoplasms on their general body metabolism?

It seems rather interesting that Group "B" should have shown the greatest evidence of neoplastic changes in the lungs, and that these changes were unquestionable and vivid in some instances. The seven animals showing metastatic deposits in the lung apparently reacted differently over the same period, which can probably be based upon the assumption of varying resistances rather than upon size or location of the primary growth.

Group "D", which showed five cases of metastases in the lungs was again interesting when making a comparison with Group "B"; however, No. 116 was of especial interest by virtue of the size and overall involvement of the lung tissue.

It would appear that even though the incidence of the primary growth does not vary from the control groups, the degree of metastases does vary considerably, with, as has been stated, a more profound metastasis in Group "B". Again, all show the inflammatory condition regardless of the group and it is assumed that had the animals in Group "D" continued to survive they could well have caught up with Group "B".

It is very difficult to say whether the cells in the secondary areas are transported through the tissues to the lungs, since in every instance the primary tumor could be shelled out from its attachment to the thoracic wall. When the lungs were carefully examined, no evidence of adhesions were noticed during the autopsy of any animal. The other question that comes to mind is that concerning methylcholanthrene itself. Does it exert its influence via the circulation? This is difficult to prove because in order to arrive at a satisfactory conclusion one would have to ignore any local application and study lung response alone. Are these metastases <u>true</u> metastases? Naturally the question can only be determined when a greater number of animals are used.

Throughout this research the word METASTASIS appears quite consistently. Its use does not signify that secondary manifestations result from the primary lesions any more than the primary lesions could be considered secondary from the lung neoplasms. One can be specific and say that in many of the animals examined squamous cell carcinomas and adeno-carcinomas were found but their source of origin is undetermined. Whatever characteristics the lung neoplasms bear to the skin neoplasms is difficult to evaluate irrespective of any similarity in the microscopic appearances. Another important point to consider is the absence in the research of a <u>normal</u> factor.

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Colum.
Drs. Kolin, Clum
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Wilson

TOBACCO INDUSTRY RESEARCH COMMITTEE

#267

APPLICATION FOR SUPPORT OF RESEARCH PROJECT

Date: February 19, 1960

1. Name of Investigator: Christopher M. Martin, M. D.

2. Title: Assistant Professor of Medicine, and Director, Division of Infectious Diseases

3. Institution and Address:
Seton Hall College of Medicine
24 Baldwin Avenue
Jersey City 4, New Jersey

4. Project or Subject: INTERACTIONS OF VIRUSES AND SUBSTANCES IN TOBACCO SMOKE CONDENSATE

5. Detailed Plan of Procedure:

It is proposed to study, in vitro and in vivo, the interactions of common human respiratory tract viruses and substances present in tobacco smoke. It is planned to study specifically (1) the degree to which whole virus or infectious viral nucleic acid will bind substances in crude condensates of tobacco smoke; (2) the effects in susceptible small animals and in tissue cultures of, respectively, virus alone, virus and concurrently administered smoke condensate, smoke condensate alone, and virus pre-treated with smoke condensate.

If clear-cut evidence of virus-smoke condensate interactions is obtained, or if various combinations of virus and smoke condensate induce unusual cellular necrotizing, inflammatory, or proliferative effects suggestive of synergism, then one or more of the following lines of investigation will be followed: (1) Fractionation of the condensate to determine the nature of the interacting substance; (2) attempts to duplicate interactions using already identified components of smoke condensate (trace metals, nicotine, polycyclic hydrocarbons, aldehydes, etc.); (3) Attempts to block synergistic effects (in animals) by prior immunization of the animal against the virus involved.

(A) Binding of Smoke Condensate substances by Viruses in vitro:
High titer suspensions of the following respiratory and oropharyngeal viruses infectious for man will be prepared: Influenza A and B; Hemadsorption Viruses Types I and II; Herpes simplex; Coxsackie A_9 and B_1; Adenoviruses Types 1, 3, and 7; JH virus; Coe virus. Each will be equilibrated at 37° C. and at refrigerator temperature with solutions of crude smoke condensate, and the mixtures sedimented in a Spinco Model L Ultracentrifuge. The resulting sediments and supernatants will be examined and compared with those of unequilibrated control suspensions by paper chromatographic methods, spectrophotometry, spectrofluorometry, fluorescence microscopy, and trace metal analysis of ashed aliquots.

Similar binding experiments using infectious viral nucleic acid of low protein content will be performed.

(B) Interactions in vivo:
The effects of virus alone, of virus and concurrently administered smoke condensate, of smoke condensate alone, and of virus pre-equilibrated with smoke condensate will be examined in the following systems:

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Virus	Animal, Route of Infection	Tissue Culture
Influenza A	Mouse, intranasal	Monkey kidney, Chick chorioallantois, Mouse lung. Human embryonic tracheal epithelium, lung, kidney
Hemadsorption Virus I & II	---	Human embryonic kidney, Monkey kidney
Herpes Simplex	Mouse, intranasal, Hamster, intraoral	Human embryonic kidney, trachea, lung
Coxsackie A_9	---	Human embryonic kidney
Coxsackie B_1	Young adult mice, intranasal, intraperitoneal	" " "
Adenovirus	---	Human embryonic kidney

6. Proposed Budget:

	1st Year	2nd Year
Salaries	$3,000	3,000
Expendable supplies	2,000	2,000
Permanent Equipment	5,000	2,000
Overhead	750	750
Other	--	--
Total	$10,750	7,750

7. Anticipated duration of work:
2 years: April 1, 1960 - March 31, 1962

8. Facilities and Staff available:
Facilities: Remodeling of the permanent laboratories of the Division of Infectious Diseases, located in the Center Building of the Jersey City Medical Center, is proceeding well ahead of schedule. Nine rooms, encompassing 2100 square feet of floor space, have been assigned to the Division. Remodeling of 6 rooms (1600 sq.ft.) in the form of two offices, three laboratories, and a glassware preparation room, will be completed April 1, 1960. Spacious animal quarters are available on top floor, same building. Equipment includes freezer, refrigerator, incubator, storage, and other tissue culture facilities; extensive fume hood space; centrifuges, spectrophotometer, automatic precision balance. Radioisotope equipment, chromatography equipment, and a muffle furnace will be purchased with funds awarded in support of USPHS Research Grant E-3257 -- see below.
Personnel: Investigator, 2 technicians, secretary, glassware diener, and, starting July 1, 1 research fellow (one year) and two medical students (3 months each).

9. Additional Requirements (Explanation of Budget Plan):
(a) Salaries ($6,000.-) - major support of one technician for two years.
(b) Permanent equipment ($7,000.-) portion of cost of Spinco Model L Ultracentrifuge ($3,000.-) and of Aminco Spectrophotofluorometer ($3,000.-).

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10. Additional information (including relation of work to other projects and other sources of supply):

(A) Relation of Work to other projects:

The project proposed in this application represents a specific application of several broad concepts to be studied along fundamental lines under USPHS Research Grant E-3257, "Transferrin-Virus-Metal-Carcinogen-DNA Interactions", for which the Division of Infectious Diseases has been awarded $18,724 for the first year of study.*

Topics being studied under E-3257 include:

(a) Nature of the transferrin-sensitive reaction in viral synthesis.
(b) Trace metals in virus replication and nucleic acid formation.
(c) Physiologic intracellular function of transferrin.
(d) Properties, mode of action of transferrin-like antiviral compounds.
(e) Carcinogen-metal complexes and their affinity for nucleic acids.
(f) Carcinogen-binding by viruses.

(B) Background of proposed study:

Data developed in the past two years at Harvard Medical School and reported to the American Society for Clinical Investigation in May, 1959 indicate that transferrin (iron-binding protein) profoundly depresses the synthesis of viral nucleic acid by infected cells through intracellular binding of trace metals other than iron (copper and manganese)[1]. Subsequent studies have demonstrated the intimate association of trace metals with viral nucleic acid chains; preliminary binding studies suggest that in the presence of trace metals viruses become extremely reactive chemicals, binding avidly to nucleic acids and to polycyclic hydrocarbons.

These observations appeared relevant to certain disparate reports by others; Rous and Friedewald in 1944[2] and Duran-Reynals in 1952[3] described erratic synergistic effects between viruses and organic carcinogens. Many investigators, in attempting to produce lung tumors or chronic bronchitis experimentally, using tobacco smoke condensates, e.g. Leuchtenberger, C., et.al.(1958),[4] have noted that most effects occur only after massive exposure to the condensates, and that, in any event, metaplastic or other changes occur in a relatively non-reproducible way, not clearly related to intensity or duration of exposure. Again, in commenting on the carcinogenic properties of certain polycyclic hydrocarbons identified in tobacco smoke condensate in extremely low concentration, Wynder and Hoffman[5] conclude that "initiating" or "promoting" substances must be invoked or assumed to assign these hydrocarbons a major etiologic role in tumorigenesis.

*For background and details of previous studies conducted at the Thorndike Memorial Laboratory, Harvard Medical School, kindly refer to our earlier grant request submitted to the Committee, dated October 6, 1959, and to the copy of USPHS Research Grant Application E-3257 appended to that request.

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(C) Significance of Proposed Research:

The present study, exploring the specific relationship between tobacco smoke, common respiratory viruses, and inflammatory and proliferative disease of the lung is designed to examine the possibility that viruses may serve as vectors for delivering toxic substances in tobacco smoke to intracellular loci, thereby initiating or triggering chronic disease.

If such interactions can be satisfactorily demonstrated, they would carry the implication that chronic inflammatory or proliferative disease of the lung is preventable (through virus vaccines) and that minute quantities of toxic substances in tobacco smoke are relatively tolerable.

1. Martin, C. M., and Jandl, J. H., Inhibition of virus multiplication by transferrin, J. Clin. Invest., 38: 1024, 1959.
2. Rous, P., and Friedewald, W. F. The effect of chemical carcinogens on virus-induced rabbit papillomas, J. Exp. Med. 79: 511, 1944.
3. Duran-Reynals, F. Studies on the combined effects of fowl pox virus and methylcholanthrene in chickens, Ann. N. Y. Acad. Sci., 54: 977, 1952
4. Leuchtenberger, C. et al. Correlated histological, cytological, and cytochemical study of tracheobronchial tree and lungs of mice exposed to cigarette smoke: Bronchitis with atypical epithelial changes in mice exposed to cigarette smoke, Cancer 11: 490, 1958.
5. Wynder, E. L., and Hoffmann, D. A study of tobacco carcinogenesis: VII. The role of higher polycyclic hydrocarbons. Cancer, 12: 1079, 1959.

Signature Christopher M. Martin, MD
Director of Project

Business Officer of Institution Joseph F. Salerno
Joseph F. Salerno, Comptroller,
Seton Hall College of Medicine

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Committee:
Kotin, Chm.
Jacobson
Reimann

#253

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street, New York 17, N. Y.

Application for Research Grant

Date: October 6, 1959

1. Name of Investigator: Christopher M. Martin, M. D.#

2. Title: Assistant Professor of Medicine, and Director, Division of Infectious Diseases

3. Institution & Address: Seton Hall College of Medicine
24 Baldwin Avenue
Jersey City 4, New Jersey

4. Project or Subject: INTERACTIONS OF VIRUSES, CARCINOGENS, AND NUCLEIC ACIDS

Specific Aims: As a major corollary to virological studies described in USPHS Research Grant Application E-3257, "Transferrin-Virus-Metal-Carcinogen-Interactions,"* it is proposed to explore the several fundamental implications in the field of carcinogenesis arising from the "transferrin effect" on viral synthesis, as recently described by Martin and Jandl*. Specifically, it is proposed to study (1) the binding of carcinogens and carcinogen-metal complexes to nucleic acids; and (2) carcinogen-binding by viruses and its relation to induction of neoplasia.

5. Detailed Plan of Procedure:

A. Carcinogen-metal complexes and nucleic acids:

Selected mutagenic organic carcinogens of varying degrees of metal affinity will be studied by equilibrium-dialysis and differential centrifugation techniques, and their rate of binding to human DNA, RNA, cell nuclei, other cell fractions, and plasma proteins compared, in the presence and absence of chelating agents. Binding affinities in the presence of various trace metals will be studied, particularly those metals shown to be both nucleic-acid associated and carcinogenic (nickel, chromium).

B. Carcinogen-binding by Viruses:

The uptake of fluorescent organic carcinogens by viruses and by viral nucleic acid will be studied by the techniques noted above and a

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Copies of Curriculum Vitae attached.
* Copies attached.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

variety of tissue culture and animal systems explored with a view to demonstrating the possible role of viruses or viral nucleic acids as vectors for the delivery of carcinogens to susceptible intracellular loci. Depending upon the results of *in vitro* affinity studies, various virus-carcinogen pairs will be administered to appropriate animal hosts, and the tumor-producing efficiency of virus plus carcinogen separately, virus-carcinogen complex, virus alone, and carcinogen alone will be compared with suitable controls. Particular attention will be given to respiratory tract viruses (influenza, adenoviruses, hemadsorption viruses). Tissue culture systems will include human embryonic kidney, lung, and tracheo-bronchial epithelium. For the present, animal studies will be limited to mice and hamsters.

C. Sources of tissues:

Human embryonic tissues derived from spontaneously aborted fetuses up to 4 months gestational age, suitable both for tissue culture and for cell fractionation studies, will be available through the Obstetrics and Gynecology Department of the Margaret Hague Maternity Hospital at the Jersey City Medical Center.

D. Metal determination techniques:

See enclosed USPHS E-3257, page 5.

6. Budget Plan:

	1st Year	2nd Year
Salaries	$6,000	$7,000
Expendable Supplies	3,000	2,000
Permanent Equipment	5,000	1,000
Overhead 15% (1)	1,350	1,350
Other	-	-
Total	$15,350	$11,350

(1) Excluding permanent equipment item.

7. Anticipated Duration of Work:

2 years: January 1, 1960 - December 31, 1961

8. Facilities and Staff Available:

January 1, 1960 - May 1, 1960: Temporary laboratory in Department of Microbiology, Seton Hall College of Medicine, fully equipped to conduct virus research (freezer, refrigerator, incubator, storage facilities; spacious animal quarters shared by pre-clinical departments; adequate space for ultracentrifugation studies with Spinco Model L and for equilibrium dialysis studies; inadequate space for metals analysis studies). Permanent laboratory facilities (estimated cost $39,000) under construction at this time.

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June 1, 1960 - December 31, 1961: Permanent facilities in the Jersey City Medical Center completed, consisting of 2 virology-bacteriology laboratories; 1 chemistry laboratory with 12-foot fume hood, extensive bench space, centrifuges, spectrophotometer, automatic precision balance, plus equipment applied for in USPHS E3257.

Personnel: January 1, 1959 - June 30, 1959: Investigator, 2 technicians, secretary, glassware diener.

July 1, 1959 - December 31, 1960: Investigator; 2 Research Fellows (Dr. Clyde Wu - present address: Boston City Hospital, Boston 18, Mass.; Dr. Regina McCormack - present address: University of Virginia Hospital, Charlottesville, Va.); 2 technicians, secretary, glassware diener. (Bulk of support for this group to come from USPHS E3257 and 2 USPHS post-doctoral fellowships.)

9. Additional Requirements: (Explanation of Budget Plan)

1) Personnel - $13,000 - major portion of salaries of 1 technician and secretary for 2 years.

2) Permanent Equipment: $5,000 - including portion ($3,000) of cost of Spinco Model L Ultracentrifuge and of Zeiss Spectrophotometer ($2,000).

10. Additional Information (including relation of work to other projects and other sources of supply):

A. Background of Studies:

The proposed studies of virus-carcinogen-nucleic acid interactions represent a logical extension of increasing evidence that (1) Viral nucleic acid is an extremely reactive substance, chemically; and (2) Viral infections can be viewed as repeated assaults on the basic cellular genetic apparatus by mutagenic substances.

The long-term implications of such repeated interactions of cell and virus nucleic acid, interactions which occur primarily in youth - the period of rapid cellular growth - have barely been studied.

B. Previous Work Done on This Project:

See USPHS E3257, pages 8-9.

C. Results Obtained by Others:

The large body of evidence that viral nucleic acid is the component which endows viruses with cell- (and nuclei-) invasive properties has been reviewed by Colter (1). The most striking recent demonstration that the nucleic acid of common human enteroviruses possesses such properties, which the protein components merely modify, has been reported by Schaffer and Mattern (2).

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Following the fundamental studies of Zinder and Lederberg (3) on transduction in Salmonella, many workers, particularly in the field of bacteriophage, have demonstrated the mutagenic properties of viral nucleic acid; these data have been reviewed by Luria (4). The indirect evidence gathered by Lederberg (5) supporting the concept of such phenomena occurring in animal cells has been extended by Benzer (6) and received striking support from the studies of Ebert (7) who has demonstrated the ability of Rous sarcoma virus to act as a vector in carrying exogenous, genetically active tissue DNA into chick embryo cells.

The intimate association of trace metals with nucleic acids has recently been quantitated and partially defined by Wacker and Vallee (8); studies by several investigators on the effect of trace metals and of chelating agents on the integrity of nucleic acid chains and protein-nucleic acid bonds in nucleoproteins have been summarized by Kirby (9).

The mutagenic properties of chemical carcinogens, first demonstrated by Tatum in Neurospora (10), have since been amply confirmed; progress in relating carcinogen action to alterations in specific gene loci has been reviewed and extended by Barratt and Tatum (11).

Scattered evidence for metal-carcinogen interaction has been summarized by Furst (12). Although suggestive evidence of interaction and synergism between viruses and chemical carcinogens was adduced as early as 1944 by Rous and Friedewald (13) and in 1952 by Duran-Reynals (14), there have been no published systematic studies of such interactions in biochemical terms. Stanley (15) has speculated that mutagenic carcinogens might induce neoplasia-stimulating properties in otherwise innocuous viruses or proviruses present in cells in the carrier state.

REFERENCES

1. Colter, J.S. Nucleic acid as the carrier of viral activity. Chapter in Progress in Medical Virology, Vol. I, Ed. by Berger, E. and Melnick, J.L. Hafner, New York, 1958, pp. 1-35.

2. Schaffer, F.L. and Mattern, C.F.T. Infectivity and physiochemical studies on RNA preparations from highly purified poliomyelitis and Coxsackie viruses. Fed. Proc. 18: 317, 1959.

3. Zinder, N.D., and Lederberg, J. Genetic exchange in Salmonella. J. Bact. 64: 679, 1952.

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6. Benzer, S. The Elementary Units of Heredity. Chapter in The Chemical Basis of Heredity, Ed. McElroy, W.D. and Glass, B., Johns Hopkins Univ. Press, 1957, pp. 70-93.

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Source: https://www.industrydocuments.ucsf.edu/docs/ffvm0000

7. Ebert, J.D. (Carnegie Institute, Baltimore) Studies on Rous sarcoma virus. Paper presented at seminar, Harvard Medical School, Dept. of Bacteriology and Immunology, February, 1959.

8. Wacker, W.E.C., and Vallee, B.L. Chromium, manganese, nickel, and other metals in RNA. Fed. Proc. 18: 345, 1959.

9. Kirby, K.S. A new method for the isolation of desoxyribonucleic acids: Evidence on the nature of the bonds between desoxyribonucleic acid and protein. Biochem. J. 66: 495, 1957.

10. Tatum, E.L. Chemically induced mutations and their bearing on carcinogenesis. Ann. N.Y. Acad. Sci. 49: 87-97, 1947.

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12. Furst, A. Chelation and cancer - a speculative review. Symposium on Metal-Binding in Medicine. Philadelphia, May 6-8, 1959. To be published (J.B. Lippincott).

13. Rous, P. and Friedewald, W.F. The effect of chemical carcinogens on virus-induced rabbit papillomas. J. Exp. Med. 79: 511, 1944.

14. Duran-Reynals, F. Studies on the combined effects of fowl pox virus and methylcholanthrene in chickens. Ann. N.Y. Acad. Sci. 54: 977, 1952.

15. Stanley, W.M. Relationships, established and prospective, between viruses and cancer. Ann. N.Y. Acad. Sci. 71: 1100-1113, 1958.

D. Discussion:
Significance of this Research:

Persistent failure by many investigators in many systems to isolate a human "cancer virus" necessarily raises the question as to whether such a virus exists at all. On the other hand, there is abundant evidence, both in animals and in man, that a large number of chemical carcinogens will induce neoplasia. At the same time, however, efforts to demonstrate the etiologic role of proven environmental carcinogens in induction of neoplasia in man are in virtually all instances (G.I. tract, lung, liver) open to the objection that while experimentally exceedingly large doses of carcinogens are required to induce cancers, each of the suspect environments (artificial food dyes, industrial fumes, gasoline fumes, cigarette smoke, etc.) contains exceedingly minute quantities of carcinogen.

The present study proposes to explore a relatively plausible set of conditions, susceptible to controlled experimental analysis, which might square the negative virologic data and the discrepant carcinogen data; i.e., that minute amounts of carcinogens - not ordinarily of mutagenic significance - may induce neoplasia if bound to any of several common viruses - not ordinarily carcinogenic - and thereby delivered to the chromosomes of cells the virus invades but does not destroy.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

The evidence that such interactions can occur is summarized above. If true and widespread, these interactions would carry the implication that neoplasia so induced is preventable (through virus vaccines) and that minute quantities of environmental carcinogens are relatively tolerable.

Signature /s./ Christopher M. Martin, M.D.
Director of Project

/s./ Charles L. Brown, M.D.

Dean
Seton Hall College of Medicine

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

R: REDACTED MATERIAL

CHRISTOPHER MICHAEL MARTIN

BORN: REDACTED

PRESENT ADDRESS: REDACTED

MARITAL STATUS: REDACTED

EDUCATION AND DEGREES

1949	A.B. Harvard College
1953	M.D. Harvard Medical School

POSITIONS

1953-54	Medical Internship, II and IV (Harvard) Medical Services, Boston City Hospital
1954-56	Senior Assistant Surgeon, U.S. Public Health Service, National Institutes of Health, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland
1956-57	Assistant Resident, II & IV (Harvard) Medical Services, BCH.
1957-59	Research Fellow, Thorndike Memorial Laboratory, Boston City Hospital, Infectious Diseases Division, Maxwell Finland, M.D., Director
1959-	Assistant Professor of Medicine, and Director, Division of Infectious Diseases, Seton Hall College of Medicine, Jersey City, New Jersey

MEDICAL SOCIETIES

REDACTED

HONORARY SOCIETIES AND SPECIAL AWARDS

REDACTED

REDACTED

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PUBLICATIONS

1. Martin, C.M., Chapman, S.S., and Eaton, M.D. Concentration and purification of influenza virus, using zinc salts. Abstracts, Soc. Amer. Bacteriologists, 54th Annual Meeting, Pittsburgh, May 6, 1954.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

2. Martin, C.M., Gordon, R.S., and McCullough, N.B. Acquired hypogammaglobulinemia in an adult. Report of a case, with clinical and experimental studies. New Eng. J. Med. 254: 449, 1956.
3. Martin, C.M., Gordon, R.S., Felts, W.R., Jr., and McCullough, N.B. Studies on gamma globulin. I. Distribution and metabolism of gamma globulin and antibodies in hypogammaglobulinemic patients. J. Lab. & Clin. Med. 49: 607-616, 1957.
4. Martin, C.M., Waite, J.B., and McCullough, N.B. Antibody protein synthesis by lymph nodes homotransplanted to a hypogammaglobulinemic adult. J. Clin. Invest. 36: 405-421, 1957.
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11. Martin, C.M., Jandl, J.H., and Finland, M. Transferrin and infection. I. Transferrin levels in agammaglobulinemia; Antibacterial properties of transferrin. In preparation.
12. Martin, C.M., Jandl, J.H., and Finland, M. Transferrin and infection. II. Inhibition of virus multiplication by transferrin. In preparation.
13. Martin, C.M., and Kinnamon, A. Transferrin and Infection. III. Antiviral properties of certain metal-binding compounds. In preparation.

GRANTS AND FELLOWSHIPS

USPHS E-1695 (Harvard) 1957-58 -- Co-investigator (Maxwell Finland, M.D., Principal Investigator).
USPHS E-1695 (C1) (Harvard) 1958-59-- " " " " "
Research Fellowship of Medical Foundation of Metropolitan Boston, Inc., 1958-59.
USPHS E-3257 -- Application submitted July 1, 1959: "Transferrin-Virus-Metal-Carcinogen-DNA Interactions", Assigned to Virology and Rickettsiology Study Section; Activation Date requested: June 1, 1960.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Par A 6 c

6. Budget Plan.

ludes CASI and Group Insurance	Salaries (Two Technicians)	* $ 7,872.00
full time employees	Expendable Supplies	2,400.00
	Overhead (10%)	425.00
	Other	874.00
		500.00 (travel)
er support of two beds, if monies re available.	Total	$12,071.00

Date: February 7, 1955 00.00

7. Name of Investigator, Title and Years Work

Leonard E. Meiselas M.D., M.S., F.A.C.P.

2. Title and Staff Available

A) Asst. Attend. Physician, Dept. of Med., Maimonides Hosp., Bklyn Clinical Instructor in Medicine, State University of New York, College of Medicine at New York City.

3. Institution & Address

B) Consultant, Norman Heimann, M.D., Research Chemist to the Maimonides Hospital of Brooklyn.

C) Maimonides Hospital - 4802 Tenth Avenue, Brooklyn 19, N.Y., State University of New York, College of Medicine at New York City, Brooklyn, New York

4. Project or Subject:

1. To determine whether there is an abnormal steroid pattern in the tobacco patient compared with suitable controls.
2. To determine the metabolic pathways of compound E and Compound F in cancer patients, heart disease patients, tobacco users, and normal controls.
3. To determine whether Aldosterone is a normal or abnormal metabolic of E and F.
4. To determine whether the presence of an abnormal functioning liver is related to the production of Aldosterone.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed) or sources of supply:

Dobriner has established that an abnormal steroid pattern can be demonstrated in the urine of some patients well in advance of the clinical detection of cancer. It has also been demonstrated that compound E and F may have ameliorating effects on the biology of certain tumors. Thus it appears certain that steroid metabolism is in some way intimately associated with the problem of neoplasia either causally or subsequently.

It has also been demonstrated that Aldosterone may be found in the urines of patients with congestive heart failure. No similar studies are available in the cancer patient. In addition, there is no information to our knowledge about the metabolic pathways of compounds E and F in the cancer patient, the heart disease patient, and the normal, with particular reference to the use of nicotine. In addition, the possibility that an abnormal steroid metabolism may exist has only been briefly studied. The possibility that Aldosterone may be part of an abnormal steroid response of the host either endogenously or in response to exogenous steroid has occupied our time and efforts for the past year. We have been able to set up successful analytical chromatography for the separations of compounds E and F, Tetrohydro E and Tetrohydro F. In addition, chromatographic systems for the separation of Aldosterone and Compound E have been developed. Bioassay studies in Adrenalectomized animals are in progress.

Director of Project

Appropriate patients, reflecting the presence or absence of a positive tobacco history, presence or absence of cancer or heart disease will be admitted to the study beds of the Medical Services of the Maimonides Hospital of Brooklyn. Twenty-four hour urines will be collected, and plasma samples will be simultaneously prepared. Baseline excretions of compounds E and F, Tetrohydro E and F and 17 Ketosteroids

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will be determined by paper chromatography. Aldosterone will then be separated chromatographically from compound E and the final determination of the former will be done by bioassay of the eluate in the adrenalectomized rat against DOCA standards.

Cortisone and Hydrocortisone will be administered orally in increasing doses to suitable patients. Urinary steroid excretions and simultaneous plasma levels of 17 Hydroxycorticoids will become three time a week. Data will be collected to determine the amount of steroid excreted, excreted unchanged, or conjugated.

Blood and urinary sodium, chlorides and body weight will be determined daily. If and when edema occurs, urines will again be studied by paper chromatography for the presence of Aldosterone. If the latter is found, further studies on liver function will be performed by usual laboratory methods and by biopsy wherever possible.

The biochemical results in relation to metabolic pathways and abnormal steroid formation will be reviewed in the light of the patient's history with regard to smoking or non-smoking. The choice of patients will be made in such a fashion as to provide a suitable control series, e.g. males, females, smokers and non-smokers. It should be emphasized at this point that one of the patients that Dobriner reported an abnormal steroid in advance of clinical cancer was a patient with carcinoma of the lung. This disease will be the first of the type of cancer that will be studied in the above protocol.

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6. Budget Plan:

	Per Annum
Salaries (Two Technicians)	* $ 7,872.00
Expendable Supplies	2,400.00
Permanent Equipment	425.00
Applied Overhead (8.17%)	874.00
Other	500.00 (travel)
Total	$12,071.00
Date	** $7,300.00

*Includes OASI and Group Insurance for full time employees

** For support of two beds, if monies are available.

7. Anticipated Duration of Work:

Two years

8. Facilities and Staff Available:

A) Modern research laboratories, including chromatographic equipment, Beckman Spectrophotometer, Hood space, Centrifuge, and Flame photometer.

B) Research Assistant with B.S., M.S.

C) Research Consultant, Norman Weissman, Ph.D., Research Chemist to the Maimonides Hospital and Director of Chemistry Laboratories at Maimonides Hospital of Brooklyn.

9. Additional Requirements:

One additional junior technician.
Support for expendable supplies.
Assumption of present assistant's salary.
** The support by grant of two beds would greatly facilitate admission of the necessary gross section of patients.

10. Additional Information (Including relation of work to other projects and other sources of supply):
(Attach Plan of Procedure (use reverse side if additional space is needed)

This work has been supported completely by the Maimonides Hospital Research Funds for the past eighteen months.

Approved _____
Dean, State University of New York, College of Medicine at New York City

_____ Signature
Director of Project

_____ Business Officer of the Institution

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TOBACCO INDUSTRY RESEARCH COMMITTEE #220

150 EAST FORTY SECOND STREET

NEW YORK 17, N.Y.

Application for Research Grant

Date: November 29, 1958

1. Name of Investigator: James G. Miller, M.D., Ph.D.

2. Title: Professor of Psychiatry and Psychology, and Director, Mental Health Research Institute

3. Institution & Address: University of Michigan
Ann Arbor, Michigan

4. Project or Subject: The behavioral effects of smoking under stress.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): Only a precise knowledge of all aspects of the smoker's reaction to cigaret smoking will allow for an effective evaluation of his overall benefits or losses. This experiment is proposed to make an objective behavioral examination of cigaret smoking's possible psychological effects which allow smokers to cope more effectively and more enduringly with difficult, stressful situations.

Experimental Design: Heavy smokers (2 packs or more a day) and light smokers (1 pack or less a day) will be tested, each subject used as his own control in a balanced design, for the effects of normal smoking and of cigaret-deprivation on performance under stress. The stress situation will be a suitable modification of apparatus used successfully by Rodnick, Ferguson, and Holliday, Dille and Duffy, both in eliciting stress reactions and in demonstrating tranquilizing effects of some of the new psychoactive drugs. Essentially, while under controlled presentation of stresses (shocks, airblasts, loud noises) and threatful anticipation of these stresses, the subject is asked to perform continuously at a task requiring rapid and fine coordination (e.g., tracking a moving target).

Twenty subjects will be tested in each group: (a) 20 heavy smokers, and (b) 20 light smokers. Each subject will be seen and tested twice, at the same time on both days, with an interval of 1 to 3 days between test sessions. A test session will consist of the following: (a) training to a level of steady performance on the apparatus; (b) a baseline determination of performance; (c) a two-hour cigaret-deprivation period, during which the subject will be forbidden cigarets; (d) the stress-testing session. On one day the subject will be allowed to smoke one cigaret immediately before the stress-testing session and during breaks in the session; on the other days, he will not.

Data Analysis: Overall performance, accuracy, errors, and type of errors are all measured in the stress test. The group groups will first be compared for baseline performance. This will allow for characterizations of the two populations from which these groups are samples. Changes in performance under the two conditions-- deprivation and smoking-- will be computed for each individual. The significances of the differences in these changes will be tested

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

(by t-tests for correlated means, using the IBM 650 electronic computer) for each of the groups taken separately and (if there is no significant difference between the two groups) for all smokers combined.

6. Budget Plan:

Salaries	$ 6,100
Expendable Supplies	100
Permanent Equipment	900
Overhead	2,500
Other (subject fees)	400
Total	$10,000

7. Anticipated Duration of Work: Six months, from receipt of grant until completion of the written report of results.

8. Facilities and Staff Available: Adequate testing space available in Ann Arbor. An experienced electronics engineer and staff of technicians will be available as necessary for help with the apparatus. We also have access to an IBM 650 computer. Several stress test apparatuses have already been built and pretested with psychoactive drugs, and these will be available for pretesting and use, if indicated, in this experiment. Leonard Uhr, Ph.D., Research Associate, Mental Health Research Institute, will act as co-investigator. He will be assisted by several research assistants who are currently working on behavioral experiments with psychoactive drugs.

9. Additional Requirements: None.

10. Additional Information (Including relation of work to other projects and other sources of supply): Dr. Donald G. Marquis, Ph.D., formerly at Michigan and now at the Social Science Research Council, will act as consultant to this project. Dr. Marquis has discussed with Dr. Clarence C. Little the type of behavioral experimentation that might be fruitful for testing smoking effects, and this proposal has grown out of these discussions.

We have been engaged for several years in continuing research on psychoactive drugs including Miltown, Compazine, Suavitil; on an antihistamine, Theruhistin; and on alcohol, and have developed a number of instruments and techniques for behavioral testing of patients under normal conditions and under these drugs.

Check should be made payable to the industrial consulting organization through which we work, Behavioral Science Research, Inc., and mailed in care of Dr. James G. Miller, Director, Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan.

Signature /s/ James G. Miller
Director of Project

/s/ John Bouthilet
President, Behavioral Science Research, Inc.

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Epidemiology, Statistics and Psychosomatic
Committee:
Dr. Wilson, Chairman
Dr. Cattell
Dr. Little
(Dr. Tagiuri)

#285
cf. #220
Activated 6/1/59

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East 42nd Street New York 17, N.Y.

Application for Research Grant

Date: May 16, 1960

1. Name of Investigator: James G. Miller, M.D., Ph.D.

2. Title: Professor of Psychiatry and Psychology
Director, Mental Health Research Institute

3. Institution & Address: The University of Michigan
Ann Arbor, Michigan

4. Project or Subject: "The Effects of Smoking and Deprivation of Smoking on Information Processing Ability, Arousal and Autonomic Correlates."

5. Detailed Plan of Procedure:

A study just completed by Srivastva and myself has shown certain positive findings. Light smokers showed significantly less anxiety than either heavy smokers or nonsmokers, but nevertheless heavy smokers carried out behavioral tasks better than light smokers. There were significant differences in autonomic responses, the light smokers having higher Galvanic Skin Response scores under various experimental conditions than the heavy smokers, suggesting that the autonomic nervous systems of light smokers might be more responsive than the heavy smokers'.

We propose to follow up suggested clues in this investigation by a further research. This research would center around a new electronic apparatus recently designed and constructed by us called the IOTA (Information Overload Testing Aid). With this apparatus it is possible to measure the rate at which information can be handled by an individual under various conditions, such as while smoking and while deprived. Furthermore it is possible to alter the monotony in the information to be handled by the subject in order to determine whether in monotonous situations the individual's arousal falls, causing him to make omissions or errors or to delay his responses.

Recent work using information theory mathematics has suggested arousal may be a function of the monotony or variability in a stimulus situation. It is probable that the level of arousal is related to function of the reticular activating system of the nervous system, and may be related to the level of the Galvanic Skin Response.

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The purpose of this research then would be to determine the information processing capacity of individuals while smoking and after prolonged deprivation, as well as their accuracy and efficiency under these conditions. The amount of the monotony in the task would be varied, so that the subject's state of arousal would vary. We would test the hypothesis that while smoking smokers maintain a higher level of arousal, so that there is an increase in their information processing capacity and in their general work effectiveness. We would also test a second hypothesis that this increase in arousal is related to a higher level of Galvanic Skin Response.

Experimental Design: The subjects would be a group of regular heavy smokers. They would be trained to operate the IOTA apparatus and their baseline Galvanic Skin Response would be determined under normal conditions after several hours without smoking. They would then be given a capsule of nicotine or a placebo before undergoing a repetition of the test. The amount of nicotine in the capsule will be so regulated as to provide a definite nicotine effect in smokers of the same order of magnitud and duration as that which would be obtained by smoking a cigarette with deep inhalation. The tests would be given at the appropriate period of time after ingestion of the capsule to assure a peak nicotine effect. If the effect wears off too rapidly, the tests will be given on more than one occasion until they are all completed.

The tests administered on the IOTA apparatus would vary in degree of redundancy or monotony in the stimulus and the amount of monotony in the external environment would also be altered as an independent variable.

Changes in performance and in Galvanic Skin Response under the two conditions, deprivation and smoking, will be computed for each individual and for the group. The significance of the differences in these changes will be tested, using our electronic computer.

6. Budget Plan:

Salaries (including fringe benefits)	6,250
Expendable Supplies	800
Computer and computation service	750
Subject fees	400
Overhead (33 1/3%)	2,733
Total	$10,933

7. Anticipated Duration of Work: Eight months from receipt of grant until completion of the written report.

8. Facilities and Staff Available: Adequate testing space available in the new Mental Health Research Institute in Ann Arbor, Michigan. Electronics engineer and technicians available as necessary to maintain the apparatuses. We have access to an LGP 30 computer. The Principal Investigator will be assisted by several research assistants currently working on other behavioral investigations, including Mr. Kent Marquis.

9. Additional Requirements: None

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10. Additional Information:

This research design is developed after consideration of the findings of our previous study on which we have submitted a report to you entitled "Performance, Personality and Physiological Correlates of Smoking as Related to Stress." The IOTA apparatus is a new model of a device based on previous research by us outlined in the attached reprint "Information Input Overload and Psychopathology." These two recent investigations are part of a continuing program of study of behavioral effects of drugs, alcohol and tobacco.

The check for support of this research should be made payable to the industrial consulting organization through which we work, Behavioral Science Research, Inc., and mailed in care of Dr. James G. Miller, Director, Mental Health Research Institute, The University of Michigan, Ann Arbor, Michigan.

Signature James G. Miller
Director of Project

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Epidemiology, Statistics and Psychosomatic

TOBACCO INDUSTRY RESEARCH COMMITTEE

Committee:

Wilson, Chm.
Little
Cattell
(Tagiuri)

150 East Forty Second Street

New York 17, New York

#284

Cf. #220
Activated: 6/1/'59

Application For Research Grant

Date: July 12, 1960

1. Name of Investigator: James G. Miller, M.D., Ph.D.

2. Title: Professor of Psychiatry and Psychology, and Director of the Mental Health Research Institute

3. Institution & Address: The University of Michigan
Ann Arbor, Michigan

4. Project or Subject: "The Effects of Nicotine on Behavior, Under Stress and Normal Conditions, in Smokers and Nonsmokers."

5. Detailed Plan of Procedure:

A study just completed by Srivastva and myself has shown certain positive findings. Light smokers showed significantly less anxiety than either heavy smokers or non-smokers, but nevertheless heavy smokers carried out behavioral tasks better than light smokers. There were significant differences in autonomic responses, the light smokers having higher Galvanic Skin Response scores under various experimental conditions than the heavy smokers, suggesting that the autonomic nervous systems of light smokers might be more responsive than the heavy smokers'.

We propose to follow up this research by a further investigation to determine the behavioral effects of nicotine ingested orally upon performance in a series of behavioral tests.

Thirty or more apparently normal subjects will be used divided equally between smokers and nonsmokers. Before each battery of tests the subject will be given a capsule of nicotine or a placebo to be provided by the Tobacco Industry Research Committee. It is understood that the amount of nicotine in the capsule will be a reasonable quantity to provide definite nicotine effect in smokers and yet not enough to nauseate or otherwise incapacitate nonsmokers. At the appropriate period of time after ingestion of the capsule to assure a peak nicotine effect, the subject will be given a battery of behavioral tests. If the nicotine effect wears off too rapidly the test will be given on more than one occasion until they are all completed.

The tests will include the following: Our driving battery including the AAA Driver Trainer, the Bausch and Lomb Orthorater to measure visual function, and the steadiness test. These tests have been repeatedly used by us on subjects under various tranquilizers and other centrally active drugs so that we have comparative data on them

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for other compounds besides nicotine. We shall also use tests which concern the speed of information processing in the nervous system such as the reaction time, the estimation of the subjective second, and perhaps the IOTA apparatus in at least some subjects.

Where possible each of these tests will be given repeatedly under stress and nonstress conditions. All testing situations will be repeated under nicotine and under placebo, the order of administration of these two being randomized.

Changes in performance under the two drug conditions, as well as under stress and nonstress situations, will be computed for each individual and for the group. The significance of the difference in these changes will be tested, using an electronic computer.

6. Budget Plan:

Salaries (Including fringe benefits)	$ 6,250.
Expendable Supplies	800.
Computer and computation service	750.
Overhead (33 1/3%)	2,733.
Other (subject fees)	400.
	$10,933.

7. Anticipated Duration of Work:

Eight months from receipt of grant until completion of the written report.

8. Facilities and Staff Available:

Adequate space for testing is available in the new Mental Health Research Institute in Ann Arbor, Michigan. Electronics engineer and technicians available as necessary to maintain the apparatuses. We have access to an LGP-30 computer. The Principal Investigator will be assisted by several research assistants currently working on other behavioral investigations, including Mr. Kent Marquis.

9. Additional Requirements: None.

10. Additional Information (Including relation of work to other projects and other sources of supply): This research design is developed after consideration of the findings of our previous study on which we have submitted a report to you entitled "Performance, Personality, and Physiological Correlates of Smoking as Related to Stress." The IOTA apparatus is a new model of a device based on previous research by us outlined in the attached reprint "Information Input Overload and Psychopathology." These two recent investigations are part of a continuing program of study of behavioral effects of drugs, alcohol, and tobacco.

The check for support of this research should be made payable to the industrial consulting organization through which we work, Behavioral Science Research, Inc., and mailed in care of Dr. James G. Miller, Director, Mental Health Research Institute, The University of Michigan, Ann Arbor, Michigan.

/s/ James G. Miller
Director of Project

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

CROSS REFERENCE SHEET

Name or Subject: Norman Molomut

Regarding: Res. Grant #

SEE

David M. Spain

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TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #146 (see also #53R1) Report No. 4

Dr. Hugh Montgomery
University of Pennsylvania

November, 1958

Influence of Nicotine (i.v.) and Tobacco Smoking on Blood Flow in Human Skin and Skeletal Muscle

SUMMARY

Several methods were used to measure the response of the peripheral circulation to nicotine administered intravenously. Nicotine decreases blood flow in skin and increases it transiently in resting skeletal muscle.

METHODS

Three methods have been used in this study for measurement of blood flow in muscle.

1. Impedance plethysmograph with venous occlusion
2. Volume plethysmograph with venous occlusion
3. Hensel thermoelectric needle

At the start of the study it was felt that the only method which could be assumed to be quantitatively valid was the volume plethysmograph, but that published records of Nyboer showing parallel results from the impedance and volume plethysmograph offered a reasonable expectancy that it would be possible to determine a factor permitting conversion of results of the impedance plethysmograph into volume flows. Given a valid conversion factor the experiments could proceed using only the impedance plethysmograph and the Hensel needle, the use of the needle permitting a distinction as to whether changes in the cross section blood flows as determined by plethysmograph were due to changes in blood flow in muscle or skin. Furthermore, the impedance plethysmograph, unlike the volume plethysmograph, would allow measurements by both devices in the same calf. Unfortunately the impedance plethysmograph proved unsatisfactory.

1. The impedance plethysmograph consisted of a stable 100 kilocycle oscillator, a transformer coupled impedance bridge on AC amplifier with phase demodulator and finally an Edin DC amplifier driving one channel of a Photron direct writing oscillograph. Tow aluminum wire electrodes wet with electrode paste and secured with Elastoplast adhesive bandage encircled the ends of the limb segment being studied and conducted a fixed 100 kilocycle current to the tissue.

Between these electrodes another pair of similar electrodes picked up the voltage resulting from the passage of the current and returned this voltage to the bridge.

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In conjunction with the Edin amplifier, the stability and sensitivity of the instrument were reasonably adequate as a physical device.

As the work proceeded, it became apparent that the impedance and the volume plethysmographs, when applied to the same limb segment, were not measuring the same physiological variable. The two records following individual venous occlusions were often closely equivalent, but when the segmental blood flow had changed by various means such as Priscoline, Adrenaline, heat etc. the ratio between the two results occasionally changed in unexpected ways and sometimes in opposite directions. Furthermore, there was an unexpected difference in the nature of the artefact which usually followed occlusion. Finally there were frequent sudden changes in impedance which were not accompanied by changes in the volume record.

As a result of these discrepancies between the impedance and the volume methods it was not considered that any reliable interpretation could be placed on the impedance results, hence it was not used in later studies of drug action.

2. The volume plethysmograph finally adopted consisted of an acrylic tube 4 inches long and 5-3/4 inches inside diameter with flanged ends and a connection for 1/2 inch tubing. Rubber sleeves tailored to fit casts of a variety of legs and terminated by rubber flanges were fitted over the cuff and secured to the plethysmograph by clamping the rubber flanges of the sleeves between the flanges on the ends of the plethysmograph and lucite rings, pressure being maintained by spring clamps reaching between rings on opposite ends of the plethysmograph. In general this device is quickly applied, and, as it does not depend upon a seal between instrument and skin, there has been little trouble with leakage.

Displaced air volume, actually a change which is partially volume and partially pressure but always proportional to volume displacement, was measured by Lilly Technitrol capacitance manometer with special extremely low pressure head having a diaphragm of 4 inch diameter made of 2 mill. brass. Calibrating volumes were inserted into the system by means of a 5 ml. hypodermic syringe.

A pneumatic cuff was applied just above the knee and produced venous occlusion by inflation from a reservoir yielding a cuff pressure of approximately 55 mm.

To eliminate return flow from the foot a second pneumatic cuff was applied aroung the ankle and was inflated to 180 to 190 mm.Hg just before the start of each series of venous occlusions. The use of this distal cuff limited the length of a series of blood flow measurements. The short time interval required between successive occlusions precluded release and reinflation of this distal cuff between venous occlusions.

3. The Hensel thermoelectric needle consists of a 19 gauge steel hypodermic needle enclosing two iron-constantan thermocouples, series opposing to measure temperature differences, and an electric heater. One of the thermocouples is at the tip of the needle, the other 1 cm. away from the tip. The heater heats the tip of the needle.

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Source: https://www.industrydocuments.ucsf.edu/docs/hvm0000

As used by Hensel, the needle is heated by alternating current from the line via transformer and rheostat, and the differential temperature is measured by galvanometer or photoelectric galvanometer recorder.

As used in this study the differential temperature was measured by a Brown strip chart recorder with 10°C scale and heating was by a 1000 cycle current with a very stable oscillator, the use of the higher frequency being necessitated by the sensitivity of the Brown recorder to 60 cycle current. When used in various calibrating media such as water, glycerine, vaseline etc. the results checked with those using the 60 cycle heating current, hence the two systems are equivalent, with the Brown recorder giving the higher sensitivity.

The Hensel needle derives immediately from a similar but less satisfactory device by Grayson but ultimately from related devices from three other laboratories, Gibbs', Rhein's and Schmidt's. In refinement of design it appears to leave little room for improvement and its stability is remarkable.

The sensitivity to blood flow is due to the movement of blood carrying away heat from the heated thermocouple at the tip of the needle. Increases of blood flow result in a decreased temperature differential between the heated and unheated thermocouples.

The operation of the Hensel needle depends upon introducing it into a satisfactory location, this being defined as: a location, where, with the heat off, three minute arterial occlusions, and the subsequent reactive hyperemia, produce little or no change in the temperature difference between the two thermocouples; but where, with the heat on, a fairly good increase in differential temperature follows arterial occlusion and a still larger decrease in differential temperature follows release of a three minute arterial occlusion.

In our studies excellent results were obtained when a satisfactory site in the muscle was located but we had much more difficulty in locating satisfactory sites than Hensel's publications would imply, with the result that in over half of the experiments no results were obtained from the Hensel needle.

The final design of the experiment used in studies of the effects of nicotine on blood flow in human muscle consisted of the following procedure:

a. The left calf was iontophoresed with adrenaline 1:1000 at a pH of 3.5 and a current of 30 milliamperes producing a profound and longlasting vasoconstriction of the affected skin area well beyond that included under the plethysmograph.

b. The volume plethysmograph was applied to the left calf, a proximal pneumatic cuff was applied above the knee to produce venous occlusions and a cuff was placed around the ankle to prevent blood flow in the foot from affecting measurements.

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Source: https://www.industrydocuments.ucsf.edu/docs/fvnd0000

c. Following intracutaneous infiltration of procaine, the Hensel needle was inserted into the right gastrocnemius muscle, with heating current off. When the differential temperature became steady a proximal pneumatic cuff was inflated to 180 mm. for 3 minutes, then released. If no appreciable change of the differential temperature occurred the distal thermocouple was heated 4-5° above the proximal thermocouple and the occlusion and release was repeated. If a satisfactory response (0.3° or better) was not obtained on release of the occlusion, the needle was moved slightly and the occlusions repeated. When a satisfactory site for the tip of the needle was found the needle was ready to make measurements of blood flow for an indefinite period with no further attention.

d. The distal cuff on the left ankle was then inflated and a series of venous occlusions was started and while they were continued 6 micrograms of adrenaline was injected over 15 seconds via an intravenous drip of 5% glucose. The needle site was considered satisfactory, and was used for collection of data on nicotine, by the Hensel technique, only after a typical transient response to adrenaline was obtained. After a waiting period of 10 to 15 minutes baseline flows by both methods were recorded, and nicotine was injected I.V.; the dose in these experiments varied between 0.6 to 4 milligrams and the duration of administration was either 1 or 4 minutes. While continuous readings were obtained with the needle, venous occlusions were made repeatedly for a period of about 10 minutes, and then the ankle cuff was deflated. Plethysmographic measurements were discontinued (needle readings continued) for 10 to 15 minutes. Then the ankle cuff was reinflated and a final series of measurements by both methods was made.

In most of the experiments with 1 minute injections the use of the Hensel needle was attempted. Successful sites were obtained in nearly half the instances, and there was good correlation between these results and those by plethysmograph. In most of the experiments with 4 minute injections the Hensel needle was not used.

65 subjects were used, including those in preliminary trials.

RESULTS

1. 41 Cases (58 injections): The injection of 6 micrograms of adrenaline during 15 seconds invariably resulted in a large but very transient increase in the blood flow in the calf muscle.

2. 16 Cases: The injection of 2 milligrams of nicotine in smokers over a period of 1 minute produced a significant increase in the mean muscle flow over the period between 1 and 3 minutes after start of injection.

3. Due to spontaneous variations in blood flow and the limited number of measurements possible during the response to nicotine, the results were not significant in every individual subject but were significant for the group (i.e. young, healthy male smokers).

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

4. 3 Cases: The results for non-smokers are not clearly significant because of the small numbers of non-smokers subjected to smoking and the high frequency of nausea; however, the few individuals tested showed a greater vascular response than did the smokers.

5. In the two instances in which 2 milligrams of nicotine were injected more slowly, over a period of 4 minutes, in smokers there was no appreciable change in blood flow to muscle.

6. 18 Cases: The injection of 3 milligrams of nicotine, to smokers, over 4 minutes produced a large increase in muscle blood flow. In one case a like response was produced with 4 milligrams.

7. In no case did the injection of nicotine produce a decrease in the blood flow to the muscle. The usual result is a transient increase followed by an unstable period with intermittent small increases. The statistical significance of the instability has not yet been determined.

8. There was no significant long term effect on the muscle blood flow following nicotine injection. The trend appears to be slightly upward but it would take many more cases to establish significance.

9. In the small number of cases where reliable measurements were obtainable by the needle as well as the plethysmograph, good substantiation was obtained of the conclusion that the increase in flow occurred in muscle rather than in the adrenaline iontophoresed skin around the muscle. This conclusion was further strongly substantiated by thermal observations on digital blood flow, previously made, and by inclusion of a digital plethysmograph in some of the experiments herein reported which invariably showed a decrease of skin flow following the nicotine injection.

10. The injection of nicotine produced marked decrease in digital blood flow (plethysmograph) and in distal volume pulse with onset preceding the increase in muscle blood flow. These cutaneous vascular effects were more sustained than were the muscular.

SUMMARY OF CONCLUSIONS

Nicotine given intravenously causes a transient increase in blood flow in resting skeletal muscle.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

53

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

No comment

Salaries
Expendable Supplies
Application For Research Grant
Overhead
Other Direct Activity

Date: December 17, 1954

1. Name of Investigator: Hugh Montgomery, M. D.

2. Title: Associate Professor of Medicine, Medical School, University of Pennsylvania

3. Institution & Address: University of Pennsylvania
Philadelphia 4, Penna.

4. Project or Subject: Influence of tobacco smoking on the blood flow of skin and of muscles of extremities in sympathectomized and unsympathectomized subjects.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

I recently requested a research grant from the Tobacco Industry Research Committee for studying the influence of tobacco smoking on the cutaneous temperature and cutaneous blood flow in human extremities. The Committee has suggested studies of blood flow in muscle and skin, and the influence of tobacco smoking on these, in sympathectomized and unsympathectomized extremities. To carry out the laboratory equipment is made the studies we first [illegible] are seeking information in the removal of the [illegible] and have available experience in the [illegible]. Normal subjects and subjects with peripheral arterial disease will be studied. An attempt will be made to choose patients about to undergo sympathectomy, so they can be re-studied after recovery from sympathectomy. The flow in skin will probably be studied in toes or fingers. Satisfactory measurements of skin temperature have been made during tobacco smoking in sympathectomized and normal individuals by Rappaport et al, Circulation 2, 850, 1950. Their plethysmographic measurements are not valid because the plethysmographic cup was applied to the toe and the cuff to the ankle. For most of the range of flow, skin temperature alone is sufficient, and we will repeat this work of Rappaport to know the magnitude of the skin flow change at the time that muscle flow is also being studied. The method will be that of skin temperature in constant temperature (to 5 C) cool room. We shall resort to plethysmographic measurements of toe flow only if that is needed to detect slight changes, at the higher range of flow where skin temperature measurements are not as sensitive to flow changes. (Felder, D., Russ, E., Montgomery, H. and Herwitz, O., Clinical Science, 13, 251, 1954). [illegible] in studying the equipment.

Our initial measurements of muscle flow will be by needle thermocouple designed and built in our laboratory by Mr. George Peirce. The materials of the thermoelectric needle itself have already been accumulated. Recording by single channel Brown potentiometer will be possible as soon as that instrument can be obtained. It seems wise to bring at least one other instrument for measuring muscle flow into the study to make certain of the direction of changes in blood flow and to learn the general magnitude of the flow and of the changes in flow. For these purposes we are discussing the relative advantages of electrical impedence plethysmography and of electrical recording direct volume plethysmography, and think it best to include in the budget costs of both of these equipments.

It may be advisable to make some polarographic measurements to help substantiate the flow measurements, especially in the case of muscle. We are now wholly [illegible] equipped to make

[illegible]

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

polarographic measurements of oxygen tension in muscle as well as in skin; with extremities at complete rest and assuming no change in metabolism (oxygen uptake) of these two tissues, oxygen tension measurements may give good indication of flow. We have demonstrated this to be so in skin (Montgomery H. and Horwitz, O.: J. Clin. Invest., 39,1120, 1950) and Charles Wolferth, Jr. and Andrew Boyd, Jr. have shown a consistent relationship in dog muscle (unpublished).

1003541503

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

6. Budget Plan (per annum)

Salaries	$4750.
Expendable Supplies	500.
Permanent Equipment	3100.
Overhead	2137.50
Other Social security	190.
Total	$10,667.50

7. Anticipated Duration of Work: Two years

8. Facilities and Staff Available: 2 constant temperature rooms, multiple thermocouples with electronic recording, air plethysmographs for use on digits, apparatuses and electrodes for measuring oxygen tension in intact tissues; electrodes for use in human muscle, a modern laboratory unit for research in circulatory problems, a supply of subjects and patients.

9. Additional Requirements: An electronic expert. Two research assistants with special skill in skin temperature work and plethysmography, these to work approximately two days per week for a two year period under the Investigator's supervision.

10. Additional Information (including relation of work to other projects and other sources of supply):

I recently requested a research grant from the Tobacco Industry Research Committee for studying the influence of tobacco smoking on the cutaneous oxygen tension and cutaneous blood flow in human extremities. The Committee has suggested studies of blood flow in muscle and skin, and the influence of tobacco smoking on these, in sympathectomized and unsympathectomized extremities. We have all the necessary equipment to make the studies we first suggested, are equally interested in the proposal by the Committee, and have considerable experience in the usual methods of measuring (plethysmographically) cross-sectional blood flow in the extremities of man and in animals. However, to carry out the proposed measurements of flow in muscle, I think it is highly desirable to use a method such as Hensel's "thermoelectric needle". Dr. Hensel's work in Dr. Henry Barcroft's laboratory at St. Thomas' Hospital in London (reported 4th Macy Conference on Shock & Princeton, Dec. 1954) has shown a good correlation between changes in muscle flow (intravenous adrenalin) measured by plethysmograph and by this needle, and no correlation with the rate of the disappearance of radioactive sodium. Calibration of the thermoelectric needle is not well established. The plethysmographic method suffers, in man from including skin as well as muscle. We are inclined to think the thermoelectric needle method will be the best with which to enter the study, but to quantitate the results it will be necessary to turn to plethysmography and it may be desirable to attempt some calibrations of the needle in perfused animal muscle as well. The reason for mentioning these factors is to justify considerable additions to the budget, because of the greater cost of equipment and need for an expert (Mr. Peirce) in designing and assembling the equipment.

[illegible] thermocouple designed and built in our laboratory by Mr. Lucas Wilson. The materials of the thermoelectric needle itself have already been accumulated. Perhaps by [illegible] quantitative will be possible as soon as this instrument can be obtained. It seems wise at this time to have at least one other instrument for measuring muscle flow into the study to learn certain of the directions of changes in blood flow and to learn the general magnitude of the flow and of the changes in flow. For these purposes we are discussing the relative [illegible] Signature Hugh Montgomery and of electrical recording direct writing plethysmography, best to include in the budget each of both of these equipments.

It may be advisable to make some polarographic measurements to help substantiate the flow measurements, especially in the case of muscle.

Director of Project

Business Officer of the Institution: Norman Topping
Vice-President in Chg. of Medical Affairs

10035415O4

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan
(March 1, 1955
through Feb. 28, 1957)

Salaries
Expendable
Overhead (45%)
Other (for subjects)
(Social Security)

Renewal of
Application For Research Grant

#53 R1

	35,850.00
	1,500.00
	300.00
	2,610.00
	67.50
	44.00
Total	$40,367.00

Date: December 1, 1955

1. Name of Investigator: Hugh Montgomery, M.D.

7. Anticipated duration of work:
to March 1, 1957

2. Title: Associate Professor of Medicine, Medical School, University of Penna.

8. Facilities and Staff Available: Facilities as in original application plus the following permanent equipment, now in use: direct volume plethysmograph; [illegible] potentiometer; impedance plethysmograph.

3. Institution & Address: University of Pennsylvania
3400 Spruce St., Philadelphia 4, Penna.

Staff: Hugh Montgomery, M.D., Director of Project; George Poirce, Research Fellow, expert in electrical equipment and design; David Polcer, Research Assistant; Elliar Ruez, Research

4. Project or Subject: Influence of tobacco smoking on the blood flow of skin and of muscles of extremities in sympathectomized and unsympathectomized subjects.

9. Additional Requirements:

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):
(In addition, indicate previous duration of work to other projects and other sources of support):

Continuation of plan as submitted in our original application dated December 17, 1954. See progress report appended.

Signature: Hugh Montgomery, M.D.
Director of Project

Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/lfxm0000

1003541505

TOBACCO
330 4TH AVE.

6. Budget Plan:
(March 1, 1956 through Feb. 28, 1957)

Salaries	$5,850.00
Expendable Supplies	1,000.00
Permanent Equipment	500.00
Overhead (45%)	2,632.00
Other (for subjects)	568.00
(Social Security)	117.00
Total	$10,667.00

7. Anticipated Duration of Work:

to March 1, 1957

8. Facilities and Staff Available:

Facilities as in original application plus the following permanent equipment, now in use: direct volume plethysmograph; single channel Brown potentiometer; impedence plethysmograph.

Staff: Hugh Montgomery, M.D., Director of Project; George Peirce, Research Fellow, expert in electrical equipment and design; David Felder, Research Assistant; Ellier Russ, Research Assistant; Barbara Ross, Technician

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Continuation of plan as submitted in our original application dated December 17, 1956. See progress report attached.

None

Signature s/ Hugh Montgomery, M.D.
Director of Project

Norman Topping
Business Officer of the Institution

10035415O6

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

CONFIDENTIAL

TIRC Grant #53

Progress Report #2

Hugh Montgomery, M.D.
University of Pennsylvania
Medical School

December 1, 1955

"Influence of Tobacco Smoking on the Blood Flow of Skin and of Muscles of Extremities in Sympathectomized and Unsympathectomized Subjects"

The plan of work for the second year on the above project (see appended renewal application) has changed little from that submitted in our original application of December 17, 1954. It has become increasingly apparent that the basic quantitation of blood flow of muscle of man is dependent upon the conventional venous pressure plethysmograph. This had to be especially designed to allow simultaneous measurements by an electrical impedence plethysmograph. Consequently, the conventional plethysmograph chosen contains air rather than water. A difficult problem of minute leakage has been solved. The problem of time-consuming assembly of the combined instruments upon the leg (or forearm) has been largely solved by re-design of conventional plethysmograph fittings.

Preliminary runs of one type of plethysmograph against the other show a reasonable correlation. This will probably allow us to proceed with simultaneous measurements by the Hensel thermolectric needle and the impedence plethysmograph. If a good correlation exists between these two methods, we will probably use the thermoelectric needle for most of this work with nicotine and tobacco. If it does not, we may choose the impedence plethysmograph for at least the exploratory work.

With the impedence plethysmograph we have conducted several experiments in which intravenous adrenalin was used. In general an increase in cross-sectional (mostly muscle) flow and a decrease in cutaneous flow (skin temperature) has resulted. We intend to continue to use adrenalin or nor-adrenalin as a means of altering muscle flow in experiments designed to learn the usefulness of each of the separate methods of measurements of muscle flow. We think the final standard must be the conventional plethysmograph, but we hope to avoid this clumsy technique if one of the other two methods correlates with it well.

We are having some difficulty in obtaining the purest nicotine, and a separate letter is enclosed asking whether the Tobacco Industry Research Committee has by now a standard source of this.

Respectfully submitted,

s/ Hugh Montgomery

Hugh Montgomery, M.D.
Director of Project

1003541507

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6 Budget Year

Salaries (1 technical) 2,000.00
Expendable Supplies 1,000.00
Application For Research Grant No comment
.00
Overhead 400.00
Other .00
Date: August 6, 1954 .00

1. Name of Investigator: Philip O'Bryan Montgomery

2. Title: Associate Professor of Pathology

3. Institution & Address: Southwestern Medical School of The University of Texas
2211 Oak Lawn Avenue, Dallas 19, Texas

4. Project or Subject: The investigation of the possible role of chronic inflammation in chemical carcinogenesis.

Additional Personnel Requirements: None for first year. The direction of the project will depend upon the results of the experiments. It is anticipated that the additional personnel requirements will be between $2,000.00 and $10,000.00 per year for the next five years.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Young adult rabbits will be injected intra-pleurally with small amounts of turpentine to produce a sterile chronic inflammatory exudate. A cell-free extract of this material will be prepared by centrifugation, and daily injections and topical applications of it will be made on the ears of other rabbits. A similar material will be prepared with the addition of a small amount of Benzpyrene added to the turpentine. This amount will be accurately measured and then recalculated on a per unit volume basis by its spectral absorption at 365 mu. The inflammatory exudate will not be utilized for injection or painting until the Benzpyrene no longer gives its characteristic absorption peak. The experiment will then be repeated as outlined above.

The purpose of this investigation is to learn whether or not a carcinogenic substance - in this case Benzpyrene - can alter a sterile, chronic inflammatory exudate in such a way that the cell-free exudate becomes carcinogenic even though the carcinogen is not detectable in its original form by light absorption technics, and to learn more about the role of chronic inflamation in carcinogenesis.

Signature /s/ P O'B Montgomery
Director of Project

/s/ E. A. Call
Business Officer of the Institution

1003541508

Source: https://www.industrydocuments.ucsf.edu/docs/nvnd0000

6. Budget Plan:

Salaries (1 technician)	$3,000.00
Expendable Supplies	2,000.00
Permanent Equipment	.00
Overhead	400.00
Other	.00
Total	$5,400.00

7. Anticipated Duration of Work: 1 year

8. Facilities and Staff Available: General laboratory space, animal cages, routine tissue preparation laboratory, histochemistry laboratory, two full-time technicians (not to be paid from grant), Spinco Model L ultra-centrifuge, Ultra-violet microscope with television camera and screen, RCA electron microscope. Student fellows during summer months.

9. Additional Requirements: None for first year. The direction of the project will depend upon the results of the experiments. It is anticipated that the following year's requirements will be between $5,000.00 and $10,000.00 per year for three to five years.

10. Additional Information (Including relation of work to other projects and other sources of supply): The equipment for this laboratory, as outlined above, has been donated by private subscription. The two full-time technicians now at work and are supported by The University of Texas. The histochemical work now in progress is supported by U.S.P.H.S. Grant A-643 from the Committee on the National Advisory Arthritis and Metabolic Diseases Council. No other aid is available. The current work in the laboratory is outlined as follows: 1) A study of dermal collagen in relation to skin cancer in man and experimental animals. In this study dermal collagen is being studied by a battery of histochemical tests and by microspectroscopy. Preliminary results indicate that the collagen adjacent to human skin cancer is histochemically very different than normal and that it shows a very different absorption pattern from 250 to 320 millimicrons. This work is now being extended to methylcholanthrene carcinogenesis of the skin of mice. 2) The investigation of cortisone inhibition of normal and neoplastic growth. Preliminary studies indicate that cortisone inhibition of wound healing in rats may be completely reversed by the local application of beef amniotic fluid. Similarly, cortisone inhibition of growth in chicks may be overcome by subcutaneous injections of beef amniotic fluid. These studies are now being extended to the attempt to reverse cortisone inhibition of methylcholanthrene-induced skin tumors in mice, with beef amniotic fluid.

Signature /s/ p o'b Montgomery
Director of Project

/s/ E. A. Gell
Business Officer of the Institution

1003541509

Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

Committee:
Reimann, Chm.
Kotin
Jacobson

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-second Street
New York 17, N. Y.

#271

TIRC file

Application for Research Grant

Date: March 4, 1960

1. Name of Investigator: P. O'B. Montgomery, Jr., M.D.

2. Title: Associate Professor of Pathology, The University of Texas Southwestern Medical School.

3. Institution & Address: The University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas 35, Texas

4. Project or Subject: The Investigation of the Role of the Nucleolus in the Normal and the Malignant Cell

Beginning Date: May 1, 1960 to April 30, 1961

5. Detailed Plan of Procedure:

The purpose of this study will be to investigate the role of nucleolar function in living normal and malignant cells. The principal technics for study will be time-lapse motion pictures of living cells as demonstrated by phase contrast, interference and ultraviolet microscopy. Correlative technics used will be electron microscopy, fluorescence microscopy and histochemistry.

The nucleolus is the only morphologic entity which can be resolved in the living cell whose function is obscure. Its enlargement and duplication in malignant cells, its intimate association with mitosis, and its hypertrophy in response to carcinogenic chemicals and viruses; all point to it as a structure with a key role in the transformation of normal to malignant cells. The main feature which the technics used in this study will bring to bear on the problem is elucidation of the temporal relationships between the nucleolus and the cell's structure, function and chemistry.

The most direct biological approach to this problem consists in the removal of the nucleolus of one living cell and its introduction into the cytoplam of a second living cell. Preliminary experiments in this laboratory have demonstrated that nucleoli may be removed from one living cell and transplanted to the cytoplasm of a second living cell. Phase contrast time-lapse motion picture observations of such transplanted nucleoli show that they frequently initiate mitosis in the recipient cell. In those recipient cells in which mitosis is not initiated the foreign nucleolus is brought to a juxtanuclear position and there eventually undergoes dissolution. These preliminary experiments will be continued and amplified in

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Source: https://www.industrydocuments.ucsf.edu/docs/fvm0000

the following three directions. One direction will be the transplantation of nucleoli from malignant cells into benign cells of a similar strain. The second and third directions will consist of the recording of the results of nucleolar transplantation in homologous cells and in heterologous cells by means of time-lapse motion picture photography using interference and ultraviolet microscopy. These latter two studies are designed to elucidate the role of the nucleic acids of the transplanted nucleolus in the recipient cell and the relationship of the transplanted nucleolus to the dry protein mass of the recipient cell.

The second major technic to be applied to the investigation of the role of the nucleolus in cell function will be ultraviolet microbeam irradiation of the nucleolus of the living benign and malignant cell. For this experimental approach the ultraviolet flying spot television microscope is the most elegant tool yet devised. This instrument is capable of producing a microbeam of ultraviolet down to 1 micron square. The microbeam may be visually positioned in size and shape so that the nucleolus of the cell is continuously subjected to ultraviolet irradiation, while the remainder of the cell and the field is illuminated by visible light. The results of these observations may then be recorded by time-lapse motion picture photography. At regular intervals during the course of the experiment ultraviolet absorption images of the entire cell may be obtained, along with quantitative analyses of the density of the nucleolar absorption of ultraviolet light. These experiments are designed to add to our information on the relationship between nucleolar RNA and cytoplasmic RNA in benign and malignant cells. A complete description of the technical aspects of the ultraviolet flying spot television microscope may be found in the references of the accompanying bibliography.

The third experimental procedure to be used in the investigation of the role of the nucleolus in cell physiology will be the influence of carcinogenic compounds on the nucleolus. Drs. Endo, Aoki and Aoyama of the Japanese Cancer Institute have recently described the production of nuclear inclusions in tissue culture cells treated with the carcinogenic compound 4-Nitroquinoline N-Oxide. Through the kindness of Dr. Endo we have been provided with a supply of this compound. Preliminary experiments in this laboratory have confirmed their observations. (Gann 50, 209-217, Sept. 1959) These observations will be extended in this laboratory to elucidate the role of the nucleolus in formation of the nuclear inclusions. The development of the inclusions will be recorded by time-lapse motion picture photography with phase contrast, interference and ultraviolet microscopy. The chemical composition of the inclusions will be contrasted with that of the nucleolus by histochemical procedures. The ultra-structure of the inclusions and the nucleoli will be compared by electron microscopy.

This series of experiments on the role of the nucleolus in cell function is expected to continue for approximately five years.

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6. Budget Plan: (See attached sheet)

Salaries	$3,113.00
Expendable Supplies	7,000.00
Permanent Equipment	3,000.00
Overhead (15%)	2,117.00
Other	1,000.00
Total	$16,230.00

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

7. Anticipated Duration of Work: Five years

8. Facilities and Staff Available:

Staff: Tissue culture technician full time; laboratory aid part time; senior electronics engineer full time; assistant electronics engineer full time; Resident in Pathology supported by investigative fellowship from American Cancer Society, eight medical student assistants part time.

Facilities: Ultraviolet flying spot television microscope, micromanipulator; two phase contrast time-lapse motion picture units; one time-lapse interference motion picture unit; complete tissue culture laboratory. All of the above are available full time under the senior investigator's direction - Available in the Department of Pathology on a part time basis are: a fluorescence microscope; electron microscope and complete histochemical laboratory with their associated personnel of three full time people.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

The presently constituted laboratory is supported jointly by the Damon Runyon Memorial Fund for Cancer Research, the Atomic Energy Commission-Division of Biology and Medicine, and the Southwestern Medical Foundation. The over-all objectives of the laboratory are the development and application of new technics for the study of living cell physiology. The principal efforts are directed towards the introduction of temporal resolution into living biological systems in an effort to determine the functioning role of the nucleic acids in relationship to cell structure. Experiments designed to elucidate these points utilize ultraviolet irradiation, virus inoculation, and the introduction of specific carcinogenic chemicals into living cells. The results are recorded by ultraviolet time-lapse motion picture photography, phase contrast time-lapse motion picture photography, and interference time-lapse motion picture photography. Control studies of fixed material are carried out with electron microscopy, fluorescence microscopy and histochemistry.

/s./ P. O'B. Montgomery M. D.
Director of Project

/s./ E. A. Gell
Business Officer of the Institution
5323 Harry Hines Blvd.
Dallas 35, Texas

1003541512

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries:

Student Laboratory Assistants (hourly)	$ 3,000
OASI and WCI	113

Expendable Supplies:

Tissue culture supplies, glassware, biological supplies, film, electronic supplies, scanner tubes, chemicals, enzyme	7,000

Permanent Equipment:

Optical equipment, electronic components, power supplies, timers, camera equipment	3,000

Other:

Travel - For visitation to other laboratories, for attendance at scientific meetings	1,000
	$14,113

Overhead:

15% of Direct Costs	2,117
TOTAL	$16,230

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

R: REDACTED MATERIAL

NAME: Philip O'Bryan Montgomery, Jr., M.D.

PLACE OF BIRTH: REDACTED

HOME ADDRESS: REDACTED

EDUCATION: BS Southern Methodist University, 1942
MD Columbia University, 1945

MEMBERSHIP IN MEDICAL AND SCIENTIFIC SOCIETIES:

REDACTED

REDACTED

REDACTED

REDACTED

POSITIONS:

Internship, Mary Imogene Bassett Hospital, Cooperstown, New York, 1946
U.S. Army Captain, Resident in Pathology, Veterans Hospital, McKinney, Texas, 1946-1949
Fellow in Pathology, Southwestern Medical School, Dallas, 1950-1951
Research Assistant in Pathology and Cancer Research, Cancer Research Institute, New England Deaconness Hospital, Boston, Mass., 1951-1952
Assistant Professor of Pathology, Southwestern Medical School, Dallas, 1952
Pathologist, Parkland Memorial Hospital, Dallas, 1952-
Associate Professor of Pathology, Southwestern Medical School, Dallas, 1953-
Consultant Pathologist, McKinney Veterans Hospital, McKinney, Texas, 1952-
Consultant Pathologist, Lisbon Veterans Hospital, Lisbon, Texas, 1953-
Pathologist, Texas Children's Hospital, Dallas, 1954-1955
Pathologist, Dallas City Zoo, 1955-
Medical Examiner, Dallas County, 1955-1958
Consultant Pathologist, St. Paul's Hospital, Dallas, Texas, 1958-
Consultant Pathologist, Flow Memorial Hospital, Denton, Texas, 1958-
Consultant Pathologist, Navarro County Memorial Hospital, Corsicana, Texas, 1952-

10035415 14

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

ABSTRACTS: P. O'B. MONTGOMERY, M.D.

1. Montgomery, P. O'B. and Fogelman, M. J.: Critique of the Use of Sulfanilamide and D20 for Determination of Total Body Water in Dogs. Fed. Proc., 1950, March, 9, #1.

2. Montgomery, P. O'B. and Fogelman, M. J.: Internal Water Exchange in Normal and Hypotensive Dogs. Fed. Proc., 1950, March, 9, 40-41, Part I.

3. Montgomery, P. O'B. and Muirhead, E. E.: Histochemical Studies of Acute Vascular Lesions in Bilaterally Nephrectomized Dogs. A. J. Path., 1953 (Scientific Proceedings) May-June, 24, 590.

4. Montgomery, P. O'B. and Muirhead, E. E.: A Microspectroscopic Study of Arterioles in Benign and Malignant Hypertension. A. J. Path., 1954, 30, 639-640.

5. Booth, E., Muirhead, E. E., and Montgomery, P. O'B.: A Characterization of the "Fibrinoid" of Renal Cortical Necrosis Due to the Shwartzman Reaction. Pro. Cen. Soc. Clin. Res., 1955, 46, 797.

6. Montgomery, P. O'B.: The Use of U-V Microspectro-photographic and Phase and U-V Television Densitometry Techniques in Medical Research. Inst. Radio Engineers Conv. Records, Part 9, 1955, Medical Section, 109-140.

7. Montgomery, P. O'B., Dillon, T. and Goth, A.: The Effect of A Histamine Liberator (Compound 48/80) on Methylcholantrhene Induced Skin Carcinoma in Mice. Proc. Amer. Assoc. Canc. Res., 1956, 2, 135.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan

Salaries
Expendable Supplies
Application For Research Grant
Overhead
Other

Date: September 29, 1954

1. Name of Investigator: George E. Moore, M.D., Ph.D. and Fred W. Bock, M.S.

2. Title: Director, Roswell Park Memorial Institute

3. Institution & Address: Roswell Park Memorial Institute, Buffalo 3, N.Y. (Health Research, Inc. -- Non-profit corporation for the administration of research projects for the staff of the above named Institute.)

4. Project or Subject: An investigation of the physiological effects of direct inhalation of tobacco smoke by laboratory animals and the study of the biological response of laboratory animals to continuous ingestion of diet-tobacco product mixtures.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): Several chambers have been designed to expose small laboratory animals directly to the mainstream smoke of tobacco products. This method has the following advantages:

(1) The mainstream smoke enters the nares directly; this avoids the "fall-out" inherent in the introduction of smoke into large chamber.

(2) Uniform temperatures and rate of burning can be obtained.

(3) Ease of performing toxicity tests with various tobacco products, filter tips, etc.

(4) Adaptation to smoking animals with tracheotomies.

One such apparatus (figure 1&2) has been used for nine xx months. A uniform two-second puff of smoke is drawn into the animal chamber every minute. The interval can be varied with the use of a variable transformer. The first model (illustrated) had six xxx units. Our present model has xix eight units, each of which serve two chambers. The xxxx distance between the chamber and cigarette has been shortened to two centimeters. The experiments to date have been limited to methodology and toxicity studies of several strains of mice. There are definite xx strain differences. In general, the mice maintained a normal growth curve with exposure to the smoke of xx six cigarettes per day. Each mouse is in the chamber about one hour and thirty minutes. The mice are smoked as early in the day as possible so as to reduce the effect upon nocturnal feeding.

Smoking Machine - not too bad
Can animal refuse?
during puff?
How much smoke
How many lungs?

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5 continued--

With additional support it is proposed to:

(1) Enlarge the experimental groups of mice and include additional strains by the construction of additional smoking "machines."

(2) Include guinea pigs, hamsters and rats in similar studies with the construction of special chambers.

(3) Test tobacco products processed in different ways for their physiological reactiveness.

(4) Briefly study the biological effectiveness of cigarette filters.

(5) Investigate the new possibility of maintaining small animals with tracheotomies and exposing them to tobacco smoke.

(6) Enlarge present program involving studies of the biological response of various laboratory animals to the continuous ingestion of food-tobacco product mixtures for long periods of time.

Facilities available include animal laboratories and chemical laboratories. Other studies concerned with tobacco tar fractionation are being pursued concurrently. Staff members with wide knowledge in the fields of biophysics, biochemistry and biology are available for consultation.

The monies requested are for support in addition to that furnished by New York State.

This grant request is by Health Research, Inc., which is a non-profit membership corporation for the purpose of administering bequest funds, grants, and fellowships designated for research at Roswell Park Memorial Institute. This arrangement allows us to circumbent State financial policies. It is being used for administering grants from the American Cancer Society, U.S.P.H. and others at the present time.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
NEW YORK 17, N. Y. 320 FIFTH AVENUE

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY
350 FIFTH AVENUE

6. Budget Plan:

Application for Research Grant

Salaries	$19,120.00
Expendable Supplies	8,400.00
Permanent Equipment	760.00
Overhead	2,262.40
Other	
Total	$30,542.40

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available: Roswell Park Memorial Institute is under the Public Health Department of New York State. Its major interest is study of the etiology, diagnosis, cause and nature of malignancy.

3. Institution & Address: A full time permanent research and clinical staff numbering approximately 100 are present. Basic research units include Biochemistry-- Dr. Pressman, Chief; Biology--Dr. Hauschka, Chief; Biophysics-- Dr. Hoffman, Chief.

Ample laboratory facilities and instruments are available for general support of research problems. The Institute maintains its own animal colonies and can provide inbred animals for most studies.

9. Additional Requirements: The project requirements for this request are above and in addition to the staff presently at the Institute engaged in biological research, some of which is directly pertinent to the request at hand. The most important need is for additional personnel to care for the large number of animals to be used in experiments and for skilled machinists to aid in the construction of special smoking chambers. (See previous preliminary grant request dated September 14th forwarded to Dr. C. C. Little, Chairman.)

10. Additional Information (Including relation of work to other projects and other sources of supply): At the present time related research is being carried on at the Institute as follows:

(1) Fractionation of tobacco tar and testing of individual fractionations for carcinogenicity.

(2) Preliminary experiments concerned with the toxicity of tobacco products added to the diet of laboratory animals.

(3) Design of individual chambers for the direct smoking of small laboratory animals.

Signature of Director of Project: /s/ George E. Moore, M.D.

Director, Roswell Park Memorial Institute
Deputy Treasurer, Health Research, Inc.

Business Officer of the Institution: /s/ George E. Moore, M.D.

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Proposed Budget for One Year (from previous preliminary grant dated September 14, 1954)

I	PERSONNEL	
	(1) two machinists (part-time for 6 months)	$2,300.00
	(2) two animal attendants at $2,800	5,600.00
	(3) Senior laboratory animal caretaker $3,100	3,100.00
	(4) Principal laboratory technician $3,920	3,920.00
	(5) Research biologist (70% part-time)	4,200.00
II	PERMANENT EQUIPMENT	
	(1) Vacuum pumps (2)	410.00
	(2) Drying oven	350.00
III	SUPPLIES	
	(1) Food, sawdust, etc., for mice and rats (partial support)	3,500.00
	(2) Tobacco products (partial support)	2,400.00
	(3) Glassware, slides, cover slips, etc.	400.00
	(4) Brass tubing, gears, lucite, clock motors, etc. for construction of smoking apparatus	2,100.00
	INSTITUTIONAL OVERHEAD 8%	2,262.40
	TOTAL	$30,542.40

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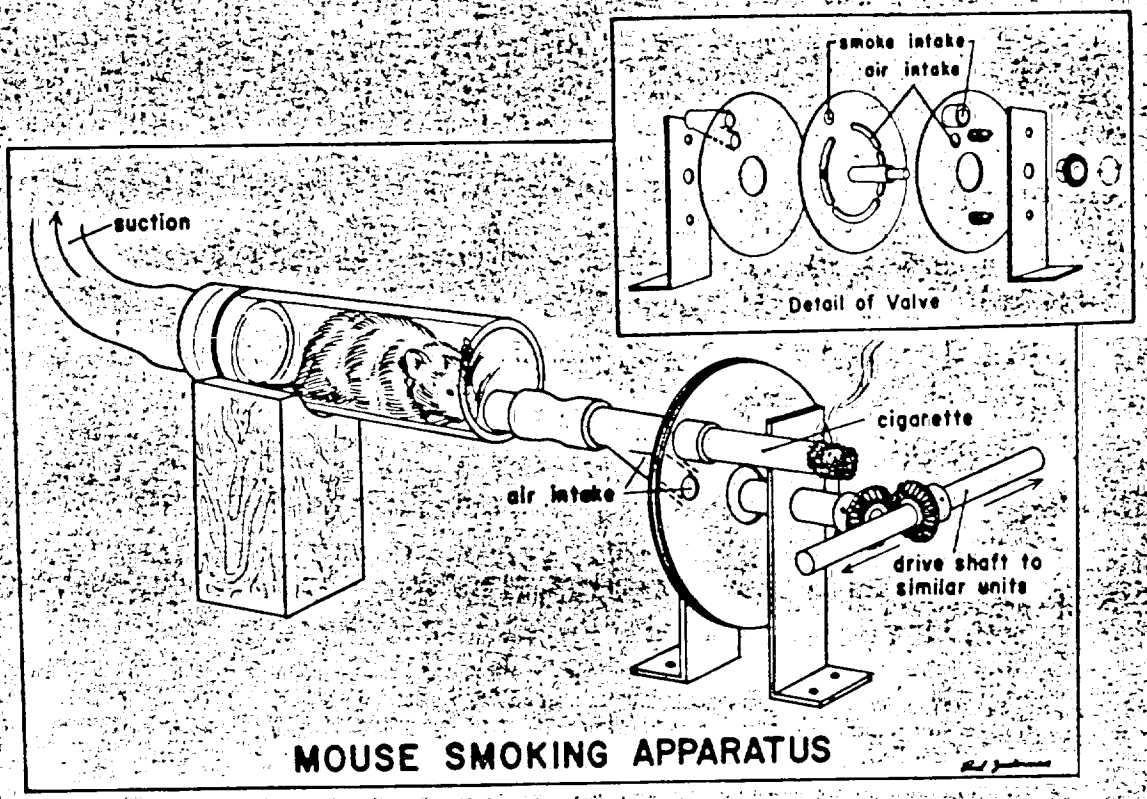

MOUSE SMOKING APPARATUS

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Source: https://www.industrydocuments.ucsf.edu/docs/hvnc0010

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan: Salaries
Expendable Supplies
Overhead (15%)
Other (Travel)

Renewal

Application For Research Grant

See notes

628 R1

Date: October 6, 1955

1. Name of Investigator: George E. Moore, M.D., Ph.D.
7. Anticipated Duration of Work: F. Bock, M.S.

2. Title:
a. Position: Dr. Moore - Director, Roswell Park Memorial Institute, Deputy Treasurer, Health Research Inc., Roswell Park Division
Mr. Bock - Senior Cancer Research Scientist

3. Institution & Address: Roswell Park Memorial Institute
666 Elm Street
Buffalo 3, New York

4. Project or Subject:

An investigation of the physiological effects of direct inhalation of tobacco smoke by laboratory animals.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed)
10. Additional information (including relation of work to other projects and other sources of support)

I. Smoke inhalation - The major activity of the program has consisted of application of cigarette smoke directly to individual mice. In the animals which have been examined to date, we have observed no tumors of the bronchial epithelium. Strong A mice which were exposed to cigarette smoke over a period of five months did not show a high level of pulmonary adenomas.

During the coming year, it is planned to set up one additional series of Swiss mice in this experiment. On the basis of our experience with this strain of mice in the apparatus now being used, the number of cigarettes smoked per day will be increased from three to ten during the first two months. At these levels, losses from acute toxicity will be small. Animals currently on the experiment will be continued at the treatment levels that have already been set up for their respective groups.

Our experience with the design of a smoking chamber for larger rodents indicates that similar instruments will be most suitable. The use of inner cones is particularly desirable for rats inasmuch as it provides for continuous growth of the animals. Accordingly, smoking machines of size sufficient for rats and hamsters will be employed to test the effect of exposure to smoke upon these animals. Such an expansion of the experiment should do much to determine whether species specificity towards cigarette smoke might be an important factor in experiment design. In this regard, the high species specificity towards the polycyclic hydrocarbons is noteworthy.

II. Studies of Crude Cigarette Smoke Condensate - Our evidence indicates that analysis of the effect of cigarette smoke condensate upon the sebaceous glands of

Director of Project

Business Office of the Institution

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mice provides a specific measure of the presence of polycyclic hydrocarbons. Within this class of compounds the relative carcinogenic activity against mouse skin matches the relative effect upon the sebaceous glands of mice. It seems probable that the materials which cause disappearance of these glands may also be responsible for the skin tumors observed in mice painted with cigarette smoke condensate.

Since species specificity is a striking characteristic in the response of not only rodents but also other mammals to skin painting by hydrocarbons, it would seem that the mouse is perhaps not the agent of choice in such analyses. Therefore, we propose to test a series of animals available to us, including rats, hamsters, guinea pigs, rabbits, dogs, and monkeys in order to determine the specificity of the response of the sebaceous glands to the application of crude cigarette smoke condensate. If whole mount technique proves suitable for animals with thick skins, the test may be extended to human subjects. The object of this phase of the investigation will be to determine the test animal of choice if differences exist.

The second phase will involve testing of various cigarettes for this general biological effect. It is planned to use three of the "American Smoking Machines" available from the Phipps and Bird Co. for this purpose. Using our present condensates, from a multiple smoking machine, we have found that the "tar" from only 40 cigarettes is sufficient for one test. Thus it becomes possible to test the material within hours of collection in order to minimize changes that might occur with storage.

This series of experiments should be sufficient to demonstrate whether there is species specificity towards the substance(s) in cigarette smoke condensate that lead to skin tumors in mice. Further, the experiments will demonstrate just how this property of smoke condensate varies with different methods of smoking, different tobaccos, and interposition of different filters or other methods of alteration of smoke composition.

III. Isolation of Fractions from Cigarette Smoke Condensate - With the development of the whole mount technique and use of serial dilution for measurement of activity against sebaceous glands, it was found possible to fractionate the smoke condensate systematically in an attempt to characterize the responsible compounds(s) responsible for this effect. Preliminary fractionation led to the conclusion that countercurrent distribution would lead to separation of the "active material" from the bulk of inactive residues. Subsequently, a 200 tube countercurrent distribution instrument was ordered by the State of New York for this project. Delivery is expected in December. With this equipment, it should be possible to concentrate the active material in quantities sufficient for bioassay with a minimum of treatment time.

IV. Studies of the Effects of Raw Tobacco in Various Diets - Our early experiments of 14 months duration have demonstrated that raw tobacco has no carcinogenic effect when fed at less than toxic levels to both mice and rats. An evaluation fa of the human experience of mouth and throat cancer among tobacco "chewers" shows that much of the positive correlation is observed in geographic areas where the general diet is poor. Accordingly, diets have been set up with deficiencies of vitamin A., thimin, and riboflavin. Dried ~~tab~~ cigarette tobacco is added in level that, in our experience, is about 1/2 of the minimum lethal dose. In one group. tobacco extracted with acetone and petroleum ether is used to control the effect of added cellulose etc. in the diets. These experiments are underway and will be continued.

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with

cased?

This is cd-not chewed

test p.e. extr aged vs unextr

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BUDGET PLAN Dec. 1, 1955 - Nov. 30, 1956

6. EXPLANATION OF SALARIES AND PERMANENT EQUIPMENT:

Salaries

Mund (Senior Medical Technician)	$3,730
Harris (Senior Animal Caretaker)	3,511
Mickens (Laboratory Worker)	2,746
Brailsford (Laboratory Worker)	2,598
Adams (Laboratory Worker)	2,598
Ramadhan (Laboratory Helper)	2,460
Bennett (Laboratory Helper)	2,236

Social Security (2%)	$19,879.00	
Workmen's Compensation (1.54%)	397.58	
	306.14	
		20,582.72

Permenent Equipment

American Smoking Machine 3 @ $1,250 — 3,750.00

PREDICATED BUDGETARY NEEDS -- Dec. 1, 1956 - Nov. 30, 1957

Salaries

Senior Medical Technician	1	$3,900
Laboratory Worker	3	8,200
Laboratory Helper	1	2,400

	$14,500	
Social Security and Workmen's Comp.	500	
		15,000

Expendable Supplies	2,250
Permanent Equipment	300
Travel	250
Overhead	2,670
	$20,470

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY
350 FIFTH AVENUE

6. Budget Plan:

Renewal — Application for Renewal

Salaries	$20,582.72
Expendable Supplies	2,250.00
Permanent Equipment	3,750.00
Overhead (15%)	4,024.91
Other (Travel)	250.00
Total Direct Cost	$30,857.63

1. Name of Investigator: George E. Moore, M.D., Ph.D.
7. Anticipated Duration of Work: F. Bock, M.S.

2 years

2. Title and Address of Institution: Director, Roswell Park Memorial Institute, Deputy Director, Health Research Inc., Roswell Park Division

8. Facilities and Staff Available:

The facilities available at the Roswell Park Memorial Institute are well adapted for the program covered by this application. Animal quarters are available for monkeys, dogs, and rodents of all types. The Institute maintains a large mouse breeding colony so that no purchases of mice is necessary. All general and laboratory supplies that are carried in the general storehouse of the Institute are available without charge against the grant. Tissue preparation is carried out by a service laboratory without charge against the grant.

In investigations of the pathological effects of direct inhala-

9. Additional Requirements:

The Institute personnel who work upon this project or directly related problems include Dr. George E. Moore, Mr. Fred G. Bock, Dr. Joseph DiPaolo, and in addition, two laboratory technicians, and five animal caretakers. Other members of the staff of the Institute have been generous in offering suggestions in their special fields.

5. General Plan of Procedure (Use reverse side if additional space is needed)
10. Additional Information (Including relation of work to other projects and other sources of supply):

I. Cigarette Inhalation - The major activity of the program has consisted of application of cigarette smoke directly to individual mice. In the animals which have been examined to date, we have observed no tumors of the bronchial epithelium. Strong a mice which have exposed to cigarette smoke over a period of five months did show a high level of pulmonary edema.

During the coming year, it is planned to set up an additional series of mice in this experiment. On the basis of our experience with this strain of mice in the apparatus now being used, the number of cigarettes smoked per day will be increased from three to ten during the first two weeks. At these levels, losses from acute toxicity will be small. Animals currently on the experiment will be continued at the treatment levels that have already been set up for their respective groups.

Our experience with the design of a smoking chamber for larger rodents indicates that similar instruments will be most suitable. The use of inner cones is particularly desirable for rats inasmuch as it provides for continuous growth of the animals. Accordingly, smoking machines of size sufficient for rats and hamsters will be employed to test the effect of exposure to smoke upon these animals. Such an expansion of the present experiment should be made to determine whether species susceptibility towards cigarette smoke demonstrated in one animal might be an important factor in experiment design. In this regard, the [illegible] cystic hyperplasia is noteworthy.

Signature
Director of Project

II. Studies of Crude Cigarette Smoke Condensate - Our evidence indicates that analysis of the effect of cigarette smoke components upon the sebaceous glands of

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: July 26th 1954

1. Name of Investigator: Burley Lee Motley, M.D.

2. Title: Professor of Medicine and Director, Cardio-Respiratory Laboratory, University of Southern California School of Medicine

3. Institution & Address: University of Southern California, School of Medicine 1212 Shatto Street, Los Angeles 17, California.

4. Project or Subject: A Study of the Effects of Smoking on Pulmonary Function

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): The purpose of the proposed project is to determine by physiological measurements the nature and extent of the changes in pulmonary function measurements which may be induced as a result of inhalation of tobacco smoke. The pulmonary function status will be evaluated by a battery of physiological tests which have been shown to have a wide range between the normal and the abnormal and to provide reliable criteria of the ability of the individual to get oxygen in and carbon dioxide out adequately both during rest and exercise. The individuals to be studied will consist of essentially normal volunteers and patients with different types of chronic pulmonary diseases both at rest and under mild to moderate stress with exercise tests employing both the step-up test and the treadmill. The method of studying the effects of smoke can be measured either before and after smoking in the usual manner and in excess and from breathing air containing tobacco smoke. The type of measurements to be obtained include lung volume measurements from spirogram recordings of total vital capacity, timed vital capacity, and maximal breathing capacity before and after bronchodilator. The residual air volume is determined by the oxygen open circuit method and duplicate checks of 100 cc. or less are obtained on each case. The alveolar nitrogen determination is made after seven minutes of oxygen breathing. The exhaled N_2 curve is obtained after one deep breath of oxygen using the N_2 meter and the electrical recorder. Resting and exercise

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tnvm0000

5 Cont.

arterial blood samples are obtained and the oxygen content and capacity and CO_2 content determined on the Van Slyke manometric apparatus in duplicate. The arterial blood pH is determined on the Cambridge glass electrode. The hemoglobin is based on the oxygen capacity and spectrophotometric. Direct tension measurements of arterial blood oxygen and carbon dioxide using the Riley bubble method are performed. Direct recordings of blood pressure from the brachial artery including recording of the pressure pulse wave with the Statham gage and electronic pressure recorder. The pulmonary ventilation measurements include resting and exercise measurements of respiratory rate, heartbeat, minute ventilation, oxygen uptake, carbon dioxide output and the percent of oxygen extracted from the inspired air breathed. Detailed studies on the distribution of inspired gases by more precise methods of measurements is desired such as afforded by a mass spectrometer permitting the use of trace gases, such as helium and carbon monoxide as indicated. The effect of bronchospasm on distribution of gases in the lungs, on pulmonary compliance and on the blood gas exchange in the alveoli appears to be an interesting aspect for investigation.

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Source: https://www.industrydocuments.ucsf.edu/docs/ftvn0000

6. Budget Plan:

TOBACCO INDUSTRY RESEARCH COMMITTEE

Salaries	$10,000.00
Expendable Supplies	3,000.00
Permanent Equipment	15,000.00
Overhead	2,000.00
Applicant's Research Grant	1,000.00
	$31,000.00

7. Anticipated Duration of Work:

Three years.

8. Facilities and Staff Available

The Cardio-Respiratory Laboratory of the University of Southern California School of Medicine is located in the Hospital of the Good Samaritan at 1212 Shatto Street, Los Angeles 17, California. This laboratory is set up and equipped for pulmonary function measurements of all types, including bronchospirometry and cardiac catheterization.

The director of the laboratory is on a full time basis and is not engaged in private practice. One assistant (M.D.) and three technicians (Cont.) over

9. Additional Requirements:

Support is requested for two additional years. It is anticipated that the ensuing two years' budgets will be approximately $20,000.00 in view of the fact that the purchase of any major items of equipment during the ensuing years will be unnecessary.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The planned project is intimately concerned with the current activities of the Cardio-Respiratory Laboratory. Pulmonary function studies are being performed daily on patients with emphysema, pneumonoconiosis, asthma, and similar medical diseases. In addition, pulmonary function studies are being carried out on pre- and post-operative patients in the field of pulmonary surgery.

Our association with the Biologic Effects of Air Pollution project as mentioned above also provides experimental and clinical subjects for the study of respiratory function following experimental and naturally occurring exposure to air pollutants. This phase of the work is being supported as part of the Air Pollution study.

Attached is the Curriculum Vitae of the principal investigator and a list of his publications.

Signature Director of Project Hurley I. Motley /s/

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

8 Cont.

are employed in the Cardio-Respiratory Laboratory and all are trained in the use of the Van Slyke in blood gas analysis and in the Scholander method of gas analysis. A full-time secretary is employed.

Included on the consultation staff is Dr. Paul Kotin and his entire staff now engaged in a full-time study of the Biologic Effects of Air Pollution. The studies include a determination of the acute, subacute, and chronic effects on animal species, clinical patients, and human volunteers.

Major Items of Permanent Equipment: 5 - Manometric Van Slykes, 3 - Scholander Gas Analyzers, 1 - Constant Temperature Water Bath Agitator and Equipment for Direct Tension Measurements of O_2 and CO_2, 1 - Cambridge pH Meter Glass Electrode, 1 - Sanborn Direct Writing Electrocardiograph, 1 - 13 liter Collins Respirometer, 2 - 120 liter Tissot type Gasometer, 2 - Sanborn Benedict-Roth type Metabolism Machines, 1 - Complete 5-channel Electrical Recorder for rates, and pressure, volume relationships (pulmonary compliance), 2 - Bennett type Intermittent Positive Pressure Breathing Units (1 - equipped with air compressor and expiratory valve housing for collecting expired air), 2 - Residual Air Combination Valve Assemblies for the oxygen open circuit method, 1 - Beckman DU Model Spectrophotometer with Fisher Power Supply, 1 - International Centrifuge, 1 - Waters Conley Oximeter, double scale unit with cuvette and earpiece, 1 - Waters Conley Nitrogen Meter, 1 - Beckman Oxygen Analyzer, 1 - portable treadmill and 1 - Fluoroscope. 1 - Liston-Becker Model 16 CO_2 Analyzer with special Esterline-Angus Recorder.

* blood pressures, electrocardiograms, stethocardiograms, airflow

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#14R2

Originally activated
June 1, 1955
Renewed as of July 1956.

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

Date: May 1, 1957

1. Name of Investigator: Hurley Lee Motley, M.D.

2. Title: Professor of Medicine and Director Cardio-Respiratory Laboratory, University of Southern California School of Medicine

3. Institution & Address: University of Southern California
3518 University Avenue, Los Angeles 7, California

4. Project or Subject: A Study of the Effects of Smoking on Pulmonary Function.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

This application is a request for the continuation of the grant started in June 1955 under a proposed three year plan on the study of the effects of cigarette smoking on pulmonary function in man. As of March 1, 1957, studies have been obtained on a group of 141 cases (all smokers) with an age range of 24 to 70 years (125 men and 16 women). Each case had pulmonary function studies including lung volume determinations and arterial blood and gas exchange measurements, except for three cardiacs and one normal case used in the compliance studies. The above investigations have been described in a paper entitled "Effect of Cigarette Smoke on Pulmonary Function Measurements" and presented before the Annual Meeting of the California Medical Association at Los Angeles, April 29, 1957. A copy of this paper has been sent direct to the Associate Scientific Director, Tobacco Industry Research Committee. During the past year data were obtained at rest in 50 cases using a smoking device so that with each breath some cigarette smoke would be inhaled in the lung with the respiratory gases. The use of the smoking device in the closed circuit insured that the smoke was distributed in the lung and should intensify the acute effects of cigarette smoke. It is desired to extend this type of study to mild exercise. Pulmonary compliance measurements have been obtained on 41 cases, using a continuous cycling method with modifications, recording volume and pressure changes on a Dumont oscilloscope with the loop interrupted by a blanking generator for time, and blips at the no flow points produced by a flow sensitive zero pressure device. The loop was photographed with a Polaroid Land Camera. Patients were studied in the sitting position during spontaneous quiet breathing, before and immediately after smoking one cigarette. There was a significant decrease in compliance after smoking in 56% of the group, while 27%

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5. showed no change and 17% demonstrated an increase in the smoking compliance. Further studies of the effect of cigarette smoking on pulmonary compliance are indicated in relation to the work of breathing in an attempt to delineate those changes due to vascular both in the greater and lesser circulation as compared to the direct effect on the respiratory mechanism. In two cases follow-up studies were obtained on emphysema subjects after smoking cigarettes had been stopped voluntarily and as far as can be determined, no medication had been taken during the non-smoking period which might be a factor in altering the pulmonary function measurements. It is hoped to obtain more follow-up studies of this type. The effect of sublingual administration of measured doses of nicotine on pulmonary function measurements is planned when the tablets are available. At the present time data on residual air measurements before and after using the smoking device, employing a simplified helium closed circuit method is under way. The helium method is rapid and checks quite closely with the oxygen open circuit method in this laboratory. No measurements have been obtained on pulmonary artery pressures while smoking as yet, but it is hoped to obtain some. This was planned on two smokers undergoing cardiac catheterization studies during the past year, but both patients developed reactions during the catheterization procedure and the smoking test was not performed. Studies are under way using the nitrogen meter and continuous recording of nitrogen wash-out for seven to ten minutes before and after using the smoking device with one cigarette. The overall studies are directed in trying to determine the nature of the changes thus far observed from inhaling cigarette smoke on lung function, whether it be bronchospasm, edema changes in the bronchiolar wall or circulatory changes especially in the pulmonary circulation.

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6. Budget Plan:

Salaries	$10,000.00
Expendable Supplies	3,000.00
Permanent Equipment	
Overhead (15.3%)	2,000.00
Other	
Total	$15,000.00

7. Anticipated Duration of Work: One more year to complete as planned.

8. Facilities and Staff Available:

The Cardio-Respiratory Laboratory is located in the Hospital of the Good Samaritan, 1212 Shatto Street, Los Angeles 17, Calif. This laboratory is equipped for pulmonary function studies of all types, including cardiac catheterization. The director, fellow, three technicians and secretary are all on a full time basis. Dr. Paul Kotin and his entire staff are consultants, who are engaged in the study of the Biologic Effects of Air Pollution.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Our association with the Biologic Effects of Air Pollution projects provides experimental and clinical subjects for the study of respiratory function following experimental and naturally occurring exposure to air pollutants. This phase of the work is being supported as part of the air pollution study.

Signature /s/ Hurley L. Motley, M.D.
Director of Project

/s/ Robert D. Fisher
Business Officer of the Institution
Financial Vice President

Source: https://www.industrydocuments.ucsf.edu/docs/...

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ROSCOE B. JACKSON MEMORIAL LABORATORY

Bar Harbor, Maine

September 18, 1958
#4

Progress Report

to

Tobacco Industry Research Committee
150 East Forty-second St.
New York 17, N.Y.

Title of Project:

The production of genetically controlled animals and tumors for possible use by T.I.R.C. grantees in experimental research on tobacco in relation to health by (a) expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids or heterozygous types as become necessary; and (c) the relation of this material to specific experimental work at the Laboratory.

Principal Investigator: William S. Murray, Sc.D.
Senior Staff Scientist and Associate Director
Roscoe B. Jackson Memorial Laboratory
Bar Harbor, Maine

Period: November 1, 1957 - September 30, 1958

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Source: https://www.industrydocuments.ucsf.edu/docs/fjvm0000

As demonstrated in the first three progress reports of work done under this grant, the breeding colonies of the listed inbred stocks of mice and their hybrids have been undergoing continuous expansion.

The rate of this increase is summarized and extended through the fourth year as Exhibit A. While this expansion has been carried on as rapidly as the breeding colonies and available housing facilities would permit, the production from the colonies has never caught up with the demand for these mice. Hence, the expansion continues through the fifth year as illustrated by Exhibit B. It is expected that this enlargement of the colony will be accomplished in the late spring of 1959.

These expansions, with the concentrations of populations which they have entailed, have created many problems in:

a) Maintenance of genetic purity of strain,
b) Health control,
c) Housing arrangements and housekeeping, and
d) Personnel training.

Genetic purity of strain has been safeguarded in the following manner:

a. Maintenance of a segregated colony of foundation stocks,
b. A carefully controlled and studied colony in which an expansion in numbers is carried on,
c. Systematic recording of the breeders obtained by the production colony from the expansion colony, and
d. Keeping animals used in final production pens as close as possible, in generations, to the original pair in the foundation stock.

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Health control has been accomplished by:

a. Systematic observation and testing of colonies for diseases indigenous to mice,
b. Systematic treatment for mite control,
c. Physical segregation of breeding colonies,
d. Rigid culling of weaklings,
e. Conversion from wooden to stainless steel breeding pens, and
f. Improved washing and sterilization techniques.

Housing and housekeeping:

The effort to find suitable space and to adapt it to our use has been continuous. This has resulted in the acquisition of five outlying buildings; arranging for heating, ventilating and waste disposal, and in the training of personnel in the necessary techniques and indoctrination in the necessary safeguards to the health of the animals.

As a result of these efforts and precautions we have been able to

Source: https://www.industrydocuments.ucsf.edu/docs/ffvm0000

keep the colonies in good health, provide breeders for renewing the old pens and for the scheduled expansion, conduct a rigid culling program, and provide others with 78% of the total production.

We are happy to report that this program is now self-supporting and that a request for further support from T.I.R.C. is no longer justified.

In addition to the above, the funds in the grant have been used to support the work of Dr. R. H. Gwynn in the "Biological activity of tobacco tars."

Dr. Gwynn's report:

During the past year the following investigations were made regarding the biological activity of Tobacco Tars*.

Effects of Tobacco tars on mouse lung and subcutaneous tissues. While there is considerable information available concerning the effects of tobacco tar on mouse skin, very little is known about its action on other tissues. For this reason the following experiments were undertaken.

a) Denicotinized tobacco tar was prepared by the method of Wynder et al. (1) and dissolved in carbowax. Small amounts of this mixture were injected into the thoracic cavities of one group of mice and into the bronchi of another. (The tar was introduced into the bronchi by means of a needle passed down the trachea.) In both cases the mice died soon after treatment. Mice treated with the solvent alone survived.

b) Lungs from 17-19 day old A/sn embryos were grafted into the axillary region of young adult A/sn mice. Two months later 40 of these grafts were threaded with silk thread which had been soaked in denicotinized tobacco tar, and 20 were threaded with untreated thread. Hosts carrying grafts with tarred threads showed symptoms of severe nicotine poisoning but recovered in a few hours. 6 months after threading hosts bearing 20 tar treated grafts were killed. No tumours were found. The remaining grafts will be examined when they have been exposed to the action of tarred threads for 12 months.

c) 0.1 ml of a mixture consisting of 2 parts of denicotinized tar and 1 part of sesame oil was injected subcutaneously into 20 A/sn mice. These were examined once each month, but no tumours have yet been found. This experiment is still in progress.

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* The Tobacco tars used in the experiments described in this report should more properly be called cigarette smoke condensates, but the shorter term is more convenient.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Effects of tobacco tars on the sterols of mouse skin. The sterols present in mouse skin may be roughly divided into two categories, Cholesterol and the "fast acting" sterols. Though small amounts of 7-dehydrocholesterol and other sterols of unknown constitution may be present, the major constituent of the "fast acting" sterol fraction is Δ^7-cholestenol (2-4), therefore, in this report the whole "fast acting" fraction will be referred to as Δ^7-cholestenol.

It has been shown by Baumann, Kandutsch and their co-workers (2-4) that the application of carcinogenic hydrocarbons to the skin of mice causes a drop in the concentration of Δ^7-cholestenol, whereas only a very few non-carcinogenic irritants have this effect. Since some workers have shown that tobacco tars are carcinogenic to mouse skin, and it is suspected that active fractions are polycyclic hydrocarbons, the effect of tobacco tar on the sterols in the skin was studied. The ultimate object of this study was to see if changes in sterol level could be used as a test for carcinogenic activity in fractions of tobacco tars.

The tar used in these experiments was made by smoking cigarettes in a simple machine. Since these experiments were of an exploratory nature no attempt was made to measure the combustion temperature of the cigarettes. The rate of smoking was two-second puffs per minute, and the yield of tar was about 4 grams per hundred cigarettes. For application to mice the tar was made up as a 50% solution in acetone (w/v). The mice used throughout these experiments were BAF_1 males, 8-10 weeks old. Tar was applied to the back, after the hair had been removed with electric clippers, using a brush which deposited 40 mg. of tar per application. Controls received acetone only. Mice were killed in various intervals after tar applications and a measured area of skin removed for sterol estimation. Total sterols were measured by the method used by Baumann (2). Sterols of experimental and control mice were always estimated at the same time. Six mice were used for each estimation. Pieces of skin adjacent of the measured area were taken for microscopical examination.

Although the concentration of Δ^7-cholestenol was always depressed by the application of tobacco tar, the degree of depression varied from experiment to experiment even when the same batch of tobacco tar was used. This appeared to be due to differences between different batches of mice, differences that require further investigation. In spite of these variations certain consistent effects were found. These are described below.

a) A single application of 40 mgs. of tar caused a depression in the concentration of Δ^7-cholestenol, the maximum depression appeared between 3 and 5 days after the application (Table 1, group a). The concentration had returned to normal by the 9th day.

b) Two applications, each of 40 mgs., given on the same day, were no more effective than a single application. (Table 1,b)

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Source: https://www.industrydocuments.ucsf.edu/docs/ffvm0000

c) Two applications, each of 40 mgs, given on consecutive days produced a greater depression in Δ^7-cholestenol concentration than did a single application and the concentration was still depressed on the 9th day. (Table 1. c)

d) Multiple applications of tobacco tar appeared to cause less depression in the concentration of Δ^7-cholestenol than did a single or two applications. When 4 applications were made over a period of 10 days and sterol content estimated on the 11th day a 24% depression in Δ^7-cholestenol concentration was found. However, when 8 applications were made over a period of 17 days no depression in the concentration of this sterol was observed. A somewhat similar observation has been made when carcinogenic hydrocarbons are applied repeatedly, but here the depression in Δ^7-cholestenol concentration persists for a greater length of time.

e) The degree of depression produced by two tobacco tars produced under different conditions was about the same. Tar (H) was produced by smoking cigarettes as rapidly as possible in the machine, while tar (S) was produced by smoking cigarettes as slowly as possible, with a degree of suction just sufficient to keep the smoke flowing into the machine. This would appear to indicate materials responsible for causing depressions in the concentration of Δ^7-cholestenol of the skin are produced even at the lowest rate of cigarette combustion. (Table 1, eH, eS).

f) The effect of Iodacetic acid, (a strong irritant for mouse skin) and β-propiolactone (a weak carcinogen) on the sterol concentration were examined. Neither caused a significant depression in the Δ^7-cholestenol concentration, although the former causes histological changes very similar to those produced by tobacco tars. (Table 1, fI, fP)

As previously stated, the degree of change in the sterol concentration after tar applications to the skin is somewhat variable. It was suspected that this might be in some way related to the state of the skin at the time of tar application. For this reason the sterol concentration of the skin was estimated at various stages in the hair cycle.

When the hair is plucked from the backs of mice during the resting phase of the hair cycle, a new cycle begins at once. Estimations of the sterol content of mouse skin taken at intervals after plucking showed that both cholesterol and Δ^7-cholestenol concentrations rose rapidly reaching a maximum at 7 days after plucking (Table 2) and thereafter declined to the normal value. Further investigation of this effect is required to discover its significance in connection with effects produced by tobacco tar.

While these experiments have yielded some interesting information

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

and suggest that changes in the concentration of 7-cholestenol may be used as a means of detecting carcinogenic activity in materials applied to the skin, considerable effort is required to elucidate the significance of this sterol in carcinogenesis.

Studies on enzyme activities in mice receiving carcinogenic and other treatments. The effect of carcinogens on enzyme systems in vivo has received little study. Apart from the importance of these systems in tumor biology, it is clear that if one or more enzymes could be shown to be specifically affected by the action of carcinogens an extremely useful means of detecting carcinogenic activity in suspect materials would be at hand. With the aim of developing such a test the effect of carcinogens on a number of enzymes in mice was investigated.

It has been shown by Adams and Roe (5) that the activity of liver catalase is depressed in mice treated with some carcinogens. Since the activity of this enzyme is also depressed by the presence of a growing tumor in the mouse, it seemed possible that other enzymes which are affected by the presence of a transplanted tumor might also be affected by carcinogens. Livers of mice bearing transplanted tumors show decreased arginase and esterase activity. Therefore, the effect of carcinogens on these enzymes as well as catalase was studied.

The carcinogens used were 9.10-dimethyl-1.2-benzanthracene, 20-methylcholanthrene and croton oil. (The last is variously described as a promoting agent or a very weak carcinogen.) A single application of each was made to the mice used. Estimations of enzyme activity in the livers were made at 2, 3 and 4 days after treatment. From these studies the following points emerged:

a) It was confirmed that mice bearing transplanted tumors showed depressed levels of all three enzyme activities.

b) Neither 9.10-dimethyl-1.2-benzanthracene nor 20-methylcholanthrene caused any depression in activity of these enzymes in the livers of the mice used in these studies. (A/sn, C57BL and BAF_1 males.)

c) Croton oil depressed the liver catalase activity of C57BL mice, and to a less extent in BAF_1 and A/sn mice. The other enzymes were not affected. (Table 3)

These results suggest that there is no direct connection between carcinogenic activity and catalase depressing action, nor is there any obvious connection between effects produced by transplanted tumors and those produced by carcinogenic materials, on the enzymes of the liver.

Experiments in progress and proposed studies.

1003541543

I) Studies on the effect of Tobacco tars on skin sterols.
II) Studies on the effect of carcinogens on enzymes of the skin and lungs.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

III) Comparison of other biochemical changes produced in mouse tissues by carcinogenic hydrocarbons and those produced by tobacco tars.

IV) Further attempts to induce tumours of the lungs and subcutaneous tissues with tobacco tars.

V) Examination of the susceptibility of sub-lines of A strain mice to the induction of lung tumours by carcinogens. (Preliminary experiments suggest that the sub-lines A/sn, A.sw, A.by and their hybrids differ considerably in their sensitivity to the lung tumour inducing action of 1.2.5.6.-dibenzanthracene).

References:

1) Wynder, E. L. and Wright, G. A study of tobacco carcinogenesis, I. The primary fractions. Cancer 10:255, 1957.

2) Miller, W. L. and Baumann, C. A. Skin sterols. IV. Distribution of Δ^7-cholestenol. Proc. Soc. Exper. Biol. & Med. 85:561, 1954.

3) Kandutsch, A. A. and Baumann, C. A. Skin sterols. VIII. Effects of carcinogens, co-carcinogens, and of certain hyperplastic agents. Cancer Research 15:128, 1955.

4) Kandutsch, A. A., Murphy, E.D., and Dreisbach, M. E. Effects of hormonal factors and of the hereditary character "hairless" on the sterols of mouse skin. Cancer Research, 16:63, 1956.

5) Adams, D. H. and Roe, F. J. C. The action of some chemical substances on mouse liver catalase activity in vivo. Brit. J. Cancer, 7:509, 1953.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TABLE 1

Effect of Tobacco tar, Iodoacetic acid and β-Propiolactone on the Δ^7-cholestenol concentration of mouse skin.

Group	Material applied	No. of applications	Total amount (Mgs.)	Concentration of Δ^7-cholestenol expressed as a % of control value this number of days after 1st application of material tested. 3	4	5	9	11
a	Tobacco tar	1	40	63	61	67	114	-
b	" "	2 on same day	80	-	52	-	-	-
c	" "	2	80	-	30	-	63	-
d	" "	4	160	-	-	-	-	76
eH	Tobacco tar H	1	40		35	-	-	-
eS	Tobacco tar S	1	40		38	-	-	-
fI	Iodoacetic acid	1	2		101	-	-	-
fP	β-propiol-actone	1	5		78	-	-	-

TABLE 2

Concentration of cholesterol and Δ^7-cholestenol at various stages in the hair cycle of mouse skin.

Days after start of cycle	Δ^7/cm^2 µg.	Δ^7% of control	Δ^5/cm^2 µg.	Δ^5% of control
3	24.1	89	104.8	150
7	70.3	260	205.0	293
12	42.8	158	145.5	208
23	27.1	100	79.9	100

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Δ^7-/cm^2 = micrograms of Δ^7-cholestenol per square cm. of skin

Δ^7% of control = concentration of Δ^7-cholestenol expressed as percentage of control value

Δ^5/cm^2 = micrograms of cholesterol per sq. cm of skin

Δ^5% of control = concentration of cholesterol expressed as a percentage of the control value

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TABLE 3

Effect of croton oil on the activity of liver catalase in mice.

Material applied	Amount applied (Mgs.)	Strain of mice	Activity of liver catalase expressed as a percentage of the control value this number of days after treatment. 2	3	4
croton oil	0.4	A/sn	93	65	115
" "	"	C57BL	43	41	50
" "	"	BAF_1	56	-	98
9.10-dimethyl-1.2-benzanthracene or 20-methyl-cholanthrene	0.2	A/sn, C57BL, BAF_1	No significant effect.		

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Exhibit A

COMPARATIVE SUMMARY MOUSE PRODUCTION

Strain	1954-55 Produced	1955-56 Produced	1956-57 Produced	1957-58 Produced
C3H	41,557	47,737	48,565	44,627
C57BL/6	6,943	40,409	107,778	209,631
DBA/1	28,006	23,471	16,585	19,335
DBA/2	57,323	63,582	88,593	139,005
BALB/c	18,202	20,972	20,449	16,255
C57BR/cd	4,808	8,590	4,562	3,624
A/Jax	17,423	18,060	21,825	20,789
A/Heston	4,140	7,496	5,610	7,260
AKR	14,659	20,743	25,829	32,653
C57 Leaden	7,432	8,559	6,327	9,237
BAF_1	3,800	11,696	13,124	17,576
CAF_1	21,342	22,391	19,463	20,903
LAF_1	21,960	29,680	28,562	26,974
BBF_1	5,008	15,463	7,409	------
C3HeB	858	3,548	2,937	3,496
$AKD2F_1$	11,075	19,137	12,918	22,793
Swiss	14,409	3,987		
ABC	454			
SWR		1,565	3,348	3,816
CDF_1	514			
RFM		3,433	5,536	3,854
CBF_1		9,940	8,027	------
BDF_1		24,403	145,187	278,897
C58		796	1,470	2,638
$D_2C_3F_1$				5,005
CBA				711
Totals	279,913	405,658	594,104	889,079

100354154?

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Exhibit B

FURTHER EXPANSION OF BREEDING COLONIES

Strain	2/28/58±	Expand to Boxes	Status Sept. 1, 1958	Each Box* Produces per year	Potential Production of Proposed Expansion
C3H	1,098	1,524	1,470	42.1	64,260
C57BL/6	3,394	6,173	4,831	57.2	353,096
DBA_1	378	378	373	56.9	21,508
DBA_2	2,472	4,212	3,805	51.0	214,812
BALB/c	280	560	336	57.0	31,920
C57Br/cd	72	84	84	58.5	4,914
A/Jax	553	553	551	37.6	20,793
A/He	224	224	224	37.0	8,288
AKR	669	787	779	54.0	42,498
C57L	218	218	224	45.3	9,875
BAF_1	168	168	168	104.7	17,590
CAF_1	245	371	315	85.3	31,646
LAF_1	315	350	259	81.7	28,595
C_3HeB	91	175	139	38.4	6,720
AKD_2F_1	217	301	217	105.0	31,605
BdF_1	3,192	3,500	3,412	101.1	353,850
SWR	70	84	70	54.5	4,578
C58	84	224	84	33.8	7,571
RFM	84	84	84	45.9	3,854
$D_2C_3F_1$	154	368	252	29.8	10,966
CBA	19	72	72	21.5	1,548
	13,997	20,410	17,749		1,270,487

* Based on records of 1957-1958

± Each box contains 2 breeding pens

100354154S

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

No Comment

Application For Research Grant

Date: December 15th, 1954

1. Name of Investigator: William S. Murray, Sc.D.

2. Title: Research Associate and Administrative Director

3. Institution & Address: Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

4. Project or Subject: The production of genetically controlled animals and tumors for use in experimental research on tobacco in relation to health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids or heterozygous types as become necessary.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The use of genetically controlled strains of animals has become an accepted principle in medical research. The employment of such controlled animal material in research projects to be initiated by, or supported by, the Tobacco Industry Research Committee has been approved as a policy by its Scientific Advisory Committee.

As a corollary of this policy, it is recognized that, if genetically "mixed" animals are to be used for some particular purpose, it will be much more satisfactory and much more adaptable to critical analysis if these heterogenous mixtures are produced in a repeatable manner by the interbreeding of known stocks.

The Jackson Laboratory has the most extensive, diversified, and complete representation of inbred mice available to medical researchers. It is therefore in a unique position to undertake the extension of existing strains, the development of new strains, or the producing of desired combinations of inter-strain hybrids. This statement applies also to the many types of tumors produced and maintained by transplantation.

Mice raised at the Jackson Laboratory have the following advantages over commercially produced animals:

1. They are obtained by expansions from carefully controlled nucleus stocks;

2. They are systematically checked for purity of strain and indications of infection;

Business Officer of the Institution

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Source: http://www.industrydocuments.ucsf.edu/docs/ffvn0000

3. They are carefully graded as to sex, age, and weight;

4. The colonies are supervised by trained geneticists, who have the cumulative knowledge and experience of some 20 resident scientists, trained also in other disciplines, readily available for consultation;

5. The genetic qualities of the inbred strains; their incidences of malignancies of various types; their morphological and physiological characteristics are supported by a 30 year accumulation of data. This accumulation of information is prehaps the greatest value of these animals, in that it enables the staff to advise scientists who are about to undertake specific problems on the selection of the animal material best adapted to provide a critical assay of their research.

At the present time the Laboratory is raising a total of 1,100,000 mice per year. Of these, 700,000 are used in the problems of its own staff and for the replenishment of the breeding colonies of the 60 inbred strains maintained and for carrying on 26 transplanted tumors. The rmak remainder are observed and studied for genetic variations and are then sold to some 400 laboratories and hospitals in 43 states and the District of Columbia. Smaller numbers are shipped to laboratories in 22 foreign countries, mxmx covering every continent.

During the last five years, the Laboratory has been faced with an ever-increasing demand for its excess animals. Requests for almost twice the number of animals the Laboratory is now able to produce are continuously on file.

Provision of facilities and personnel for the purpose of producing additional animals is an imperative need.

It is requested that the Tobacco Industry Research Committee consider a grant in support of the effort to meet this need, on the understanding that research projects supported by the T.I.R.C. will receive first consideration in the sale of the increased number of animals which will be made available.

The grant requested will by no means cover the whole cost of scientific personnel needed to supervise and check the genetic qualities of the animals studied, or of the overhead needed to provide the facilities for their production. It will, mxmkxxk however, justify the Laboratory in undertaking the production of a significant number of additional animals for the purposes mentioned. It will also make an important contribution to further and more complete genetics analysis of the various inbred strains, and their hybrids, and of the tumors which they produce both naturally and by induction. This will make the material studied increasingly valuable for all types of medical research.

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Source: https://www.industrydocuments.ucsf.edu/docs/fhyd0000

6. Budget Plan:

Salaries	$ 20,554.00	*
Expendable Supplies	7,585.00	
Permanent Equipment	9,350.00	
Overhead		
Other (Laboratory services and overhead)	9,829.00	
Total per year	$47,318.00	

*This does not include salary of Principal Investigator.

7. Anticipated Duration of Work: This is a continuing problem of parallel duration with the interest of the Tobacco Industry Research Committee in any phase of experimental research on tobacco in relation to health, which includes the use of controlled animal material to be used in vivo or in vitro.

8. Facilities and Staff Available:

The proposed plan will not require the acquisition of additional professional personnel. The laboratory is at the moment construction quarters for the proposed expansion and plans to staff these new quarters with caretakers and research assistants now in training. These will be augmented by new personnel in the unskilled categories.

The facilities, equipment and services of the Laboratory plant, valued at $1,164,000., will be available to this effort. The present machanized cleaning and sterilizing equipment has capacity for the proposed additional load.

9. Special Equipment Required:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

(Including relation of work to other projects and other sources of supply):

On the basis of past performance, this plan should make available, to research outside the Laboratory, an additional 100,000 mice per year.

Signature: William S. Murray
Director of Project

Dale J. Foley
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvh0000

1003541551

Dr. DuPuis:

I enclose also a copy of the revised application (No. 52) from Dr. William S. Murray of the Roscoe B. Jackson Memorial Laboratory. This was revised according to the recommendations of the Board at its January meeting.

This revised form was distributed on February 21st and approved by the Board.

R. C. H.

R. C. HOCKETT
Tobacco Industry Research Committee
Empire State Building
New York 1, N. Y.
LAckawanna 4-1440

1003541552

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

350 Fifth Avenue New York 1, N. Y.

Application for Research Grant

Date: December 15, 1954
(Revised copy)

1. Name of Investigator: William S. Murray, Sc.D.

2. Title: Research Associate and Administrative Director

3. Institution & Address: Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

4. Project or Subject: The production of genetically controlled animals and tumors for possible use by T.I.R.C. grantees in experimental research on tobacco in relation to health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids of heterozygous types as become necessary; and (c) the relation of this material to specific experimental work at the Laboratory.

5. Detailed Plan of Procedure: The use of genetically controlled strains of animals has become an accepted principle in medical research. The employment of such controlled animal material in research projects to be initiated by, or supported by the Tobacco Industry Research Committee has been approved as a policy by its Scientific Advisory Committee.

As a corollary of this policy, it is recognized that, if genetically "mixed" animals are to be used for some particular purpose, it will be much more satisfactory and much more adaptable to critical analysis if these heterogenous mixtures are produced in a repeatable manner by the interbreeding of known stocks.

The Jackson Laboratory has the most extensive, diversified, and complete representation of inbred mice available to medical researchers. It is therefore in a unique position to undertake the extension of existing strains, the development of new strains, or the producing of desired combinations of inter-strain hybrids. This statement applies also to the many types of tumors produced and maintained by transplantation.

Mice raised at the Jackson Memorial Laboratory have the following advantages over commercially produced animals:

1. They are obtained by expansions from carefully controlled nucleus stocks;

2. They are systematically checked for purity of strain and indications of infection;

3. They are carefully graded as to sex, age and weight;

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Source: https://www.industrydocuments.ucsf.edu/docs/nrvn0000

4. The Colonies are supervised by trained geneticists, who have the cumulative knowledge and experience of some 20 resident scientists, trained also in other disciplines, readily available for consultation. The value of supervision and study by the Laboratory Staff is apt to be underestimated. It will be recalled that statements have been made to the effect that cigarette smoking increases the probability of cancer in general. The constant investigation of the large genetically controlled colony of mice at Bar Harbor is providing increased knowledge of the etiological factors characteristic of many types of cancer. This will allow an increasingly accurate estimate of the independence and interrelationships of various sorts of neoplasms. While the degree to which such work applies to humans will remain debatable, the fact remains that relationships and principles discovered will continue to be of significance as have all phases of comparative medical research.

5. The genetic qualities of the inbred strains; their incidences of malignancies of various types; their morphological and physiological characteristics are supported by a 30 year accumulation of data. This accumulation of information is perhaps the greatest value of these animals, in that it enables the Staff to advise scientists who are about to undertake specific problems on the selection of the animal material best adapted to provide a critical assay of their research.

   Some idea as to the extent of this material may be given as follows. There are some sixty (60) strains of mice which are being maintained at the laboratory. These differ in the indicence of adenocarcinoma of the lung from 85 ± % in the highest strains to 0.1% in the lowest. Lung tumors may be isolated as the only type of neoplasm in some strains and occur in conjunction with other neoplasms in others. Data are being collected continuously on the "natural" incidence of lung tumors in these strains. Such data would be significantly augmented by the proposed grant.

   Additional animals produced and observed will also increase the incidence of new mutations and substrains, at least some of which should be useful in research of interest to the Tobacco Industry Research Committee.

At the present time the Laboratory is raising a total of 1,100,000 mice per year. Of these, 700,000 are used in the problems of its own staff and for the replenishment of the breeding colonies of the 60 inbred strains maintained and for carrying on 26 transplanted tumors. The remainder are observed and studied for genetic variations and are then sold to some 400 laboratories and hospitals in 43 states and the District of Columbia. Smaller numbers are shipped to laboratories in 22 foreign countries, covering every continent.

During the last five years, the Laboratory has been faced with an ever increasing demand for its excess animals. Requests for almost twice the number of animals the Laboratory is now able to produce are continuously on file.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

In the Laboratory's own research there are many problems which will be directly or indirectly aided by the proposed grant. A number of these may lead to prospects which will later be submitted individually to the Tobacco Industry Research Committee by the investigator or investigators concerned. As examples may be cited work by Dr. Kaliss on the effect of stress on tumor incidence in mice; the experiments on stress in relation to behavior, nervous stability and memory in dogs by Drs. Fuller et al; work on the influence of various carcinogens on transplants of cancer susceptible and non-susceptible lung tissue of mice by Little et al; and the approach to the successful synthesis of artificial culture media for animal tissues (Waymouth et al).

Profision of facilities and personnel for the purpose of producing additional animals is an imperative need.

It is requested that the Tobacco Industry Research Committee consider a grant in support of the effort to meet this need, on the understanding that research projects supported by the T.I.R.C. will receive first consideration in the sale of the increased number of animals which will be made available.

6. Budget Plan:

For the first year, 1955-56

Salaries	$ 20,554.00*
Expendable Supplies	7,585.00
(1) Permanent Equipment	9,350.00
(2) Overhead (at 15%)	5,623.00
Other	-
(Total)	$ 43,112.00

For Succeeding years

~~Salaries~~	~~20,554.00~~
Salaries	20, 554.00*
Expendable Supplies	7,585.00
(3) Overhead	4,220.00
	$ 32,359.00

*This does not include salary of Principal Investigator.

(1) This involves racks for cages and the necessary alterations of space for the work.

(2) This item has been reduced to conform with the general policy of the Scientific Advisory Board.

(3) This item is smaller than in 1955-56 because of non-recurrence of the "Permanent equipment" item.

The grant requested will by no means cover the whole cost of scientific personnel needed to supervise and check the genetic qualities of the animals studied, or of the overhead needed to provide the facilities for their production. It will, however, justify the Laboratory in undertaking the production of a significant number of additional animals for the purposes mentioned. It will also make an important contribution to further and more complete genetic analysis of the various inbred strains, and their hybrids, and of the tumors which they produce both naturally and by induction. This will make the material studied increasingly valuable for all types of medical research.

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Source: https://www.industrydocuments.ucsf.edu/docs/fhyn0000

7. Anticipated Duration of Work: This is a continuing problem of parallel duration with the interest of the Tobacco Industry Research Committee in any phase of experimental research on tobacco in relation to health which includes the use of controlled animal material to be used in vivo or ~~in~~ in vitro.

8. Facilities and Staff Available: The proposed plan will not require the acquision of additional professional personnel. The Laboratory is at the moment constructing quarters for the proposed expansion and plans to staff these new quarters with caretakers and research assistants now in training. These will be augmented by new personnel in the unskilled categories.

The facilities, equipment and services of the Laboratory plant, valued at $1,164,000.00, will be available to this effort. The present mechanized cleaning and sterilizing equipment has capacity for the proposed additional load.

9. Additional Requirements: None.

10. Additional Information: On the basis of past performance, this plan should make available, to research outside the Laboratory, an additional 100,000 mice per year.

Signature /s./ William S. Murray

Director of Project

/s./ Dale J. Foley

Business Officer of the Institution

1003541556

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Les Grants

TOBACCO INDUSTRY RESEARCH COMMITTEE

February 16, 1955

There is a change on your outline of individual investigators and institutions applying for grants from TIRC.

#52 - William S. Murray Sc.D., Roscoe B. Jackson Memorial Laboratory has sent in a revised application. The changes are as follows:

Subject - The production of genetically controlled animals and tumors for use in experimental research on tobacco in relation of health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids or heterozygous types as become necessary; and (c) the relation of this material to specific experimental work at the laboratory.

Amount - $43,112.00 - first year

The complete revised application will be distributed at the next meeting of the Scientific Advisory Board on January 20-21.

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1003541557

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

& Budget Item

Salaries
Expenses **Renewal of**
Application For Research Grant
Overhead
Other

$29,500.00
#52 Bl
1,200.00
1,250.00

Date: **December 6, 1955**

1. Name of Investigator: **William S. Murray, Sc.D.**
2. Title: **Research Associate and Administrative Director**
3. Institution & Address: **Roscoe B. Jackson Memorial Laboratory Bar Harbor, Maine**

4. Project or Subject: **The production of genetically controlled animals and tumors for possible use by T.I.R.C. grantees in experimental research on tobacco in relation to health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids or heterozygous types as becomes necessary; and (c) the relation of this material to specific experimental work at the Laboratory.**

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

1. To continue the planned expansion of the inbred nucleolus and nucleus outlined in our original proposal.

2. To continue the checks and controls for health and purity of strain in the nucleus and breeding colony.

3. To continue the checks and controls over the 20 or more transplantable tumors carried.

4. To continue the study of the life histories of first generation hybrids.

5. To expand the breeding colony started in 1955 to the point where it produces approximately 10,000 animals per month.

Signature: William S. Murray
Director of Project

Business Office of the Institution

1003541558

Source: https://www.industrydocuments.ucsf.edu/docs/mnw0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

6. Budget Plan:

		2
	Salaries	$29,500.00
	Expendable Supplies	4,139.00
Application for Research Grant	Permanent Equipment	1,500.00
	Overhead	4,200.00
	Other	
	Total	$32,359.00

Renewal of

Date December

7. Anticipated Duration of Work: This is a continuing problem of parallel duration with the interest of the Tobacco Industry Research Committee in any phase of experimental research on tobacco in relation to health which includes the use of controlled animal material to be used in vivo or in vitro.

8. Facilities and Staff Available: The staff to accomplish the proposed work is available and on the job. No additional facilities are needed. The new mouse room, equipped by T.I.R.C. in 1955, is completed and functioning very satisfactorily.

9. Additional Requirements: Replacement of animal cages — $1,500.00

10. Additional Information (Including relation of work to other projects and other sources of supply):

As mentioned in our Report of Progress, the timing of this effort has been fortunate, particularly in that it protects workers on T.I.R.C. projects against the pressure for mice created by the greatly expanded chemotherapy program of the United States Public Health Service.

Signature s/ William S. Murray
Director of Project

s/ Dale J. Foley
Business Officer of the Institution

Comptroller

100354155 9

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

CONFIDENTIAL

REPORT OF PROGRESS

December 5, 1955

to the

Tobacco Industry Research Committee

Period: March 1, 1955 - December 1, 1955

Title of Project: The production of genetically controlled animals and tumors for possible use by T.I.R.C. grantees in experimental research on tobacco in relation to health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids or heterozygous types as become necessary; and (c) the relation of this material to specific experimental work at the Laboratory.

Name of Investigator: William S. Murray, Sc.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

During the period of this report there has been a marked increase in the number of research workers who have become converted to a realization of the benefits to be derived from the use of controlled animal material in their experimentation. The tendency has been for them to lean more and more heavily upon the material available at the Laboratory. This is particularly true of agencies undertaking contracts in the greatly expanded chemotherapy program of the National Cancer Institute.

The expansion undertaken by the Laboratory under the auspices of the Tobacco Industry Research Committee has proved to be very fortunately timed, in that it has made it possible to meet the increasing current demands of the period and lends itself to the much greater expansion demanded by the large testing programs planned by those working in chemotherapy.

The expansion within the Laboratory has been accomplished by (1) approximately doubling the size of the colony maintained in the nucleolus; (2) using the animals produced in this way to accomplish an expansion of approximately 15% in the size of the inbred nucleus, this 15% being confined to those of the inbred stocks currently in great demand; (3) the increased productivity of the nucleus

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

has made it possible for us to stock the new animal room which was equipped under the non-recurring part of the budget of the T.I.R.C. grant.

The following table, which lists the number of animals produced in the new building, will give a picture of what we have been able to do in animal production through the support of this grant:

MONTH	NUMBER OF MICE WEANED IN NEW BUILDING
March	492
April	1122
May	2690
June	3462
July	3102
August	5246
September	3989
October	5688

This is over and above what we would have been able to do with our previous facilities.

At the present moment, the new building is operating at slightly over 50% capacity. We expect within the next year to bring it to capacity and, barring unforeseen difficulties, to produce in it animals at the rate of approximately 10,000 per month. Breeding animals on this scale makes it imperative that we continue to increase the diligence with which the animals in the nucleolus and in the nucleus are controlled, and that we make continuing studies of the various stocks in order to be able to insure the purity of strain. This is being done. In addition to this, we are also making studies of the normal behavior of a variety of first-generation hybrids, in an attempt to develop those types of animals for use in experiments to which they are adaptable.

10035541561

Source: https://www.industrydocuments.ucsf.edu/docs/tvmn0000

It has been our practice, in order to insure the purity of strain of the inbred animals sent to other laboratories and to minimize the chance of expanding sublines which vary from the standard set up for the parent strains, to confine animals sent from the Laboratory to those born in the first four generations after leaving the nucleus. The size of the nucleus thus becomes a controlling factor in the eventual number of inbred mice we are able to produce.

In some experiments, particularly those which use hosts for growing transplanted tumors, first generation hybrids have advantages over the parent stocks. Outstanding among these is the fact that many more of them can be produced within the limits of nucleus size and the control methods which we have established.

In the nucleus we are also maintaining 24 transplantable tumors of various types, are checking them frequently for the appearance of infection and taking immediate steps to clear up the contaminations which occasionally appear.

We have confidence that by continuing the program outlined above we shall be increasingly able to take care of any demands for animal material which researchers of the Tobacco Industry Research Committee may make upon us.

Of the possibilities for direct research within the Laboratory on problems of interest to the Tobacco Industry Research Committee mentioned in our proposal of December 15, 1954, we have been particularly intrigued by the influence of various carcinogens on transplants of cancer-susceptible and non-susceptible lung tissue. The techniques employed by Wynder and others in skin-painting of mice with tobacco derivatives and in transferring results thus obtained to a relationship with carcinogenicity of the lung, were considered.

It was felt that both the experimental setup and extension of the interpretation of results left much to be desired. Among the points which needed correction were the following:

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Source: https://www.industrydocuments.ucsf.edu/docs/rtvh0000

(1) Environmental variation between the skin and the pleural cavity. In order to approach more closely the temperature and other environmental conditions of the pleural cavity it was felt that the peritoneal cavity offered distinct advantages over the skin.

(2) It was also felt that lung tissue itself should be challenged by direct or circulatory contact with the applied or infected material. There is little structural or functional similarity between skin and lung.

(3) It was also felt that equal opportunity should be provided in a single host animal for the two types of lung tissue (that giving a high natural incidence of adenocarcinomas and that giving relatively little incidence) to meet the challenge presented by transplantation to the peritoneal cavity.

The techniques for making all these improvements have been successfully developed and a considerable number of mice are already being challenged by known carcinogens as a precursor to later use of tobacco derivatives prepared under standardized conditions. (See Exhibit I).

William S. Murray

1003541563

Source: https://www.industrydocuments.ucsf.edu/docs/fjvn0000

Exhibit I

LUNG GRAFT - CARCINOGEN EXPERIMENT CENSUS AS OF 12/5/55

Group II	Donor	Route	Host	♀♀ BP*	♀♀ DBA**	♂♂ BP	♂♂ DBA	Total number mice	
1	BAF_1	I.P. →	BAF_1	--	--	--	--	--	
2	B/10	I.P. →	BAF_1	13	--	19	--	32	
3	A	I.P. →	BAF_1	32	23	27	21	103	208 I.P.
4	B/10	I.P. →	B/10	--	--	--	--	--	
5	A+B/10	I.P. →	BAF_1	18	31	9	15	73	
6	BAF_1	S.C. →	BAF_1	10	--	10	--	20	
7	B/10	S.C. →	BAF_1	22	28	9	30	89	
8	A	S.C. →	BAF_1	14	66	13	35	128	324 S.C.
9	B/10	S.C. →	B/10	10	--	9	--	19	
10	A+B/10	S.C. →	BAF_1	14	21	11	22	68	
								532	I.P. and S.C.

* 3,4 - Benzpyrene I.V. (0.5mg/0.5cc/mouse) aqueous dispersion (W. E. Heston, N.C.I.).
** 1,2,5,6 - Dibenzanthracene I.V. (0.5 mg/0.5cc/mouse) aqueous dispersion (W.E.Heston, N.C.I.).
S.C.-Piece of lung placed S.C. through a small skin incision.
I.P.-Piece of lung sutured with absorbable catgut to the internal surface of the abdominal peritoneum.

Double grafts in one host (A+B/10): A lung placed on the left side and B/10 lung placed on the right side of the host.

Animals used: Inbred ♀ B/10 x ♂ A/Sn → BAF_1 offspring (age at graft: 5-7 weeks -- recorded)
Inbred (brother x sister) pedigreed lines of A/Sn and C57BL/10 ScBs maintained for the experiment

1003541564

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Bioassay, carcinogenesis and tissue culture

Committee:
Dr. Reimann, Chairman
Dr. Little
Dr. Kotin
Dr. Jacobson

#290

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forth-second Street New York, New York

Application For Research Grant

October 25, 1960

1. Name of Investigator: William S. Murray, Sc.D.

2. Title: Senior Staff Scientist

3. Institution & Address: Roscoe B. Jackson Memorial Laboratory
Bar Harbor, Maine

4. Project or Subject: Studies on Physiology of Mice of Different Ages.

The long-term goal is to contribute to our knowledge of various factors influencing tissue reactions and changes, including neoplasia, by a more extensive use of old mice than has hitherto been made. The immediate aims and plans of procedure are listed (in section 5) under six headings.

5. Detailed Plan of Procedure:

(1) Do old mice exert the same type and degree of modifying influence on transplants of tissues as do young adults?

Transplants of tumor and of normal tissue into successive transplant generations of old mice will be made under conditions similar to those described by Barrett, Hauschka, and others. The tolerance of these "modified" tissues will be studied by periodic reinoculation into the "negative" parental strain (the parental strain in which the tumor did not originate).

(2) How do old animals react to various doses of, and types of exposure to, the M.T.I. (mammary tumor inciter)? What are the influences on morbidity and mortality of various strains injected with filtrates, or receiving transplants, of various tissues and organs (with or without M.T.I.) from the same and from alien strains?

Injections of blood, and of filtrates of tissue, and transplantation of various tissues containing the M.T.I. will be made into old animals of the same

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and of alien strains. The systemic and local effects will be observed physiologically and histologically. Parallel experiments with blood, filtrates, and transplants free from M.T.I. will be conducted and the results will be compared.

(3) What is the type, rate, and sequence of spread of the M.T.I. when it is introduced locally and at various sites and ages?

Neonatal, young adult, and old mice with the M.T.I. will provide the specific tissues to be transferred to animals without the M.T.I. but susceptible to it. Specific tissues will be removed at intervals from the host animals to see whether or not they contain, and can transmit, the M.T.I.

(4) Can characteristic different degrees of activity of the M.T.I. be produced by substrains of hybrids derived from crosses into which the M.T.I. is introduced by a single animal? If so, are these differences the result of change in the agent, or of the genetic constitution of the substrains.

This is a long-term experiment, involving the establishment, and expansion to large numbers, of inbred strains of hybrids derived from a single-animal introduction of the M.T.I. In each generation, the effectiveness of the M.T.I. will be tested and compared by challenging animals without the M.T.I. but susceptible to it.

(5) Can the presence of the M.T.I. be detected by its use in combination with, or in sequence to, exposure to known carcinogens? Can these or other techniques hasten its detection, either directly, or by its influence on chemical carcinogenesis?

Agents known to influence the incidence of skin, lung, lymphatic, or leukemic neoplasia will be used in combination and in pre and post application to local and systemic injection of the M.T.I. The effects on neoplasia and on the physiology and histology of various tissues and organs will be observed and compared.

(6) Will gonadectomy, or other hormonal imbalances, induced after the reproductive period, influence the morbidity or mortality of inbred strains and their hybrids?

Operative techniques and injection will be used to produce hormonal imbalance in old animals beyond the reproductive period. They will be compared with controls receiving similar experimental challenges without hormonal modification. Populations of each sex will be separately studied.

6. Budget Plan:

Salaries	$ 8,219.00
Expendable Supplies	1,709.00
Permanent Equipment	1,390.00
Overhead (15%)	1,990.00
Other	1,950.00
Total	$15,258.00

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

7. Anticipated Duration of Work:

Three years. This request, however, is in support of the project prior to May 1, 1961 (see section 10).

8. Facilities and Staff Available:

Adequate animal room, laboratory, and office space are available. Cleaning and caretaking facilities are established. The scientific personnel will consist of the applicant in cooperation with Dr. Clarence C. Little and two laboratory assistants (not yet selected). The necessary animals are available in a great variety of inbred strains (and their hybrids) and in large numbers.

9. Additional Requirements:

These consist of modest alterations to the animal room and the installation of benches in the laboratory space. These are included in the "other" item of the budget. The equipment item in the budget includes appropriation for freeze-drying apparatus and animal room equipment.

10. Additional Information:

This project, as outline above, duplicates a request being submitted to the National Institutes of Health for support during the period May 1, 1961 - April 30, 1964. The present proposal, in support of getting the project underway, is for the period December 1, 1960 - April 30, 1961.

In essence this represents the attempt of the writer to re-enter the experimental field, in cooperation with Dr. Clarence C. Little, to take advantage of the availability of unusual animal material to supplement knowledge in a segment of the biological field which has been largely neglected.

/s/ William S. Murray, Sc.D.
Director of Project

/s/ Dale J. Foley
Business Officer of the
Institution

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TOBACCO INDUSTRY RESEARCH COMMITTEE #42R2
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.
Activated: 1/1/55
Renewed: 1/1/56
(Heath and Farnsworth)

Application For Research Grant

Date: November 28, 1956

1. Name of Investigator: Charles McArthur, Ph.D.

2. Title: Psychologist to the University Health Services

3. Institution & Address: The University Health Services
Harvard University
78 Mt. Auburn Street
Cambridge, Massachusetts

4. Project or Subject: Monograph on social and personal determinants of smoking behavior

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Proposal One involves an expenditure of $7500. Put simply, it would cover the continuation of the existing research until next June 30. That would mean the completion of the book or monograph, already partially completed, which was described in some detail in one section of the progress report given to you last summer. The work (and the staff) has been set up on an academic year basis and plans have been realized very nearly on schedule, so that some sort of stopping point might be reached at that time. What remains to be done is a good deal of generating of manuscript about existing results, some translation of the material presented in the crude form of contingency tables into more sophisticated statistics, and conclusion of the substudies of quitting and cutting down and of reactions to the publicity about smoking and cancer.

The resulting work would be in the form of a Genetic Psychology Monograph or of a small book. It would be too long for the usual journal and probably would make a tediously long series of journal articles.

A possibility that was not discussed with Dr. Hockett but that might be included in this six-month program would be some exploration of a new lead turned up by two Texas psychologists, who show a relationship between Rorschach content and the location of cancer within the body. It might be relevant to discover whether the Rorschach signs that they find related to the locus of the carcinoma are also related to smoking habits. This, in our data as now tabulated, would not be very difficult.

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Document hosted by the Legacy Tobacco Documents Library

6. Budget Plan:

Salaries -including Retiring Allowances	$6850
Expendable Supplies	100
Permanent Equipment	0
Overhead	550
Other	0
Total	$7500

7. Anticipated Duration of Work: Until June 30, 1957

8. Facilities and Staff Available:
The same as for the past years. Active workers on this project are Dr. McArthur, Miss Ellen Waldron and Mr. John Dickinson.

9. Additional Requirements:
none

10. Additional Information (Including relation of work to other projects and other sources of supply):

This would be a "close out" operation. If the Committee decides to accept Proposal Two, this proposal need not be considered, since the work outlined here could be done under the terms of Proposal Two without additional support.

There are no other sources of support for this work.

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Signature
Director of Project

Business Officer of the Institution

Source: https://www.nationalarchives.gov.uk/help/rights.htm

#272

Committee:
Dr. Wilson, Chm.
Dr. Little
Dr. Cattell
(Dr. Tagiuri)

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty-second Street
New York 17, N. Y.

(Cf. #42 - Heath-Farnsworth
Activated 1/1/55
Renewed 1/1/56
" 1/1/57-6/30/57)

Application for Research Grant

Date: March 29, 1960

1. Name of Investigator: Charles C. McArthur, Ph.D.

2. Title: The Social Mediation of Smoking Behavior

3. Institution & Address: University Health Services
Harvard University
78 Mt. Auburn St.
Cambridge 38, Massachusetts

4. Project or Subject: The social setting of smoking habits both at the time smoking started and at present, around age 40, will be investigated for members of the Study of Adult Development by means of the attached section of the Study's annual follow-up questionnaire. The analysis of this data by itself and in relation to known lifetime smoking histories is proposed. The interaction of the social mediation of smoking with the cultural and psychodynamic factors already seen in these same people will be examined.

5. Detailed Plan of Procedure: After tabulating the questionnaire items, relations among items will be sought. Types of first smoking experience, family smoking background and family attitudes toward smoking (Questions 9,1 and 2) will be compared to see if some common patterns do not emerge. If types of smoking families of origin can be categorized, the relations of these family backgrounds to lifetime smoking patterns will be examined. Types of lifetime histories have been described. Do certain kinds of smoking families generate sons who become essentially nonsmokers, moderate smokers, heavy smokers who can quit or late starters whose smoking accelerates addictively? A recent paper, Horn et alia, suggests that the beginning of such an influence of family can be discerned already during high school. How much does the family pattern influence smoking still by age 40?

The relation of the early social setting of smoking to the cultural and psychodynamic factors shown to relate to smoking behavior in these same people can now be seen. Are the cultural variables directly mediated by the family? Where anthropology was invoked was the phenomenon as simple as that when fathers don't smoke sons don't? Are psychodynamics irrelevant if we know what the consciously reportable attitudes of family and son were? Or do these factors supplement each other and interact in

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complex ways? The beautiful possibility of seeking interaction effects in an analysis of variance is open to us. What are the relative contributions of Nonsmoking Father and of Lower Middle Class Origin? Does the first just express the second? Heighten its effectiveness? Wash its effect out? Parallel effects of culture x family mediation x psychodynamics can be explored. The criteria measured can be total lifetime smoking, smoking at forty, ability to quit, or lifetime smoking curve type.

Next, the current social surround may be examined from Questions 4 and 5 and sometimes 6 and 7, depending on the content of the answers. Do contemporary social influences account for today's smoking behavior? Are there types of social surrounds of smokers? Do these types relate to present behavior? More important, do these types relate to present attitudes? Question 3 becomes, then, another criterion. To what extent does what people say now coincide with what people do? Will the parental press on the next generation be as it has been in the past?

The second step with this data is to see how present social surround interacts with original family surround in determining today's smoking behavior and attitudes. Does one determine behavior and the other attitudes? Again an analysis of variance is suggested: what are the contributions of Nonsmoking Contemporaries? Are these two effects additive? Or is an interaction effect Nonsmoking Origins x Smoking Contemporaries very real? Again, present smoking, lifetime smoking, quitting, curve or expressed attitudes may be used as criteria.

Once again, the relation of contemporary social surround to all the cultural and psychodynamic forces known to operate in these men should be examined. Take the heavy smoker with his impulsive personality. Does he seek Contemporary Smoking as a surround? Do his inner drives carry him right through Contemporary Nonsmoking surrounds that never touch him? What of conscientious nonsmoking? Is this supported by nonsmoking surrounds? Or does this trait, too, go on inner directedly and oblivious to social pressures?

The remaining questions will illuminate histories and smoking ideologies and will be used to test theories about the value systems that go with types of smoking histories. Questions 2 and 3 make an interesting pair. Questions 6 and 7 are aimed at the conscious report of nonsmokers, whose underlying dynamics were theoretically explained in earlier papers. Again, value analysis of all these openended questions should produce a typology that can be compared to smoking history and to other typologies already coded for these same persons. Interaction effects are again to be sought. For instance, many physicians have stopped smoking. Is this as simple a phenomenon as it looks to be or is there an interaction Physician x Moral Family Ideology that can be seen? Which physicians stopped? What patterns did recent publicity trigger? Is there a purely contemporary interaction effect like Physician x Smoking While Working that leads to different outcomes?

These analyses of culture x social groups x individual personality bring this study to a very broad conceptual level. No one has ever examined "Personality in Nature, Society and Culture" with such a universally

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available and easily quantifiable criterion as number of cigarettes consumed. We all say we believe that Nature, Society and Culture interact to determine human behavior but this may well be the first piece of human behavior for which the contributions of each of the three and the weight of the interaction effects between them has been calculated!

6. Budget Plan:

Salaries	$1,281.53
Expendable Supplies	-
Permanent Equipment	-
Overhead (15%)	192.23
Other	-
Total	$1,473.76

7. Anticipated Duration of Work: Ten weeks

8. Facilities and Staff Available: The budget covers 1/5 time for the principal investigator plus a full time coding clerk for ten weeks. All other resources (typists, office materials, calculating machinery) will be provided by Harvard. The rich longitudinal data and office facilities of the Study of Adult Development are the basic tools for this study.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

This work grew out of the studies earlier supported by TIRC and reported by Heath and also by McArthur, Waldron and Dickinson. The questions asked for this study filled gaps in the original data. The codings done for the earlier studies will be used in this study, so that the interaction between these new factors in smoking behavior and those previously observed can be run by a clerk. The early social factors can be related to known longterm subsequent smoking histories.

Signature Charles McArthur
Director of Project

Business Officer of the Institution

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QUESTIONNAIRE ITEMS

Section VI. TOBACCO

1. When you were a boy, did the following people smoke:

	Never	Sometimes	Often
Father	____	_____	____
Mother	____	_____	____
Sister(s)	____	_____	____
Brother(s)	____	_____	____

2. What were the attitudes of your close relatives about whether you should smoke?

3. What, if anything, will you advise your children about smoking?

4. Do the following people smoke:

	Yes	No
Your wife	_____	_____
Most of your friends	_____	_____
Most of your business associates	_____	_____

5. How much do you, (or, if you are an ex-smoker, did you) smoke under the following conditions:

During or after meals:

On social occasions:

While working with others:

While working alone:

When having nothing to do:

6. If you never smoked, why didn't you?

7. If you used to smoke, but don't now, why did you stop?

Did you find it: easy/moderately difficult/very difficult/ to give up smoking?

8. If you smoke, do you smoke:
Cigarettes: 1-5/6-10/11-19/1 to 2 packs/ 2 or more packs daily
Pipes: 1 - 2 - 3 - 4 - 5 - 6+ daily
Cigars: 1 - 2 - 3 - 4 - 5 - 6+ daily

9. To the best of your recollection, when and under what circumstances did you first take up smoking?

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#42A
(cf. Heath #42
Farnsworth #42R1
McArthur #42R2)

Application For Research Grant

Date: November 28, 1956

1. Name of Investigator: Charles McArthur, Ph. D., and a principal investigator who would be a psychiatrist to be named. (Dr. Hastings was originally proposed for this assignment but was not able to come here for it. We have one nominee in mind but have not approached him as yet. He is interested in coming here and would be a good man for the job.)

2. Title:

3. Institution & Address: The University Health Services
Harvard University
78 Mt. Auburn Street
Cambridge, Massachusetts

4. Project or Subject: Interviewing and perhaps retesting the participants in the Study whose earlier interviews and tests gave rise to the relationships reported in our existing work on smoking and personality.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):
Proposal Two involves an expenditure of $75,000 between January 1, 1957 and June 30, 1960. Put simply, this would cover an all-out effort to get good and full psychiatric interviews on the Study participants. The idea that was originally phrased in terms of Dr. Hastings' visiting the participants is now re-suggested in terms of a program without a specific man's name attached. Obviously, as good a man as possible will be employed.

Three major purposes, from the smoking and personality point of view, would be (1) the obtaining of good, recorded, verbatim material that is generated when the man discusses his smoking, (2) the obtaining of facts not now in the record that are directly relevant to the hypotheses we have formulated about smoking, (3) demonstrating the reliability of (a) the instruments that have already shown relationships to smoking and (b) the relationships themselves, when the instrument is not suspect but the trend existed fifteen years ago.

The open-ended verbatim material would be most useful in enabling us to establish the truth or falseness of our psychodynamic theories about these boys' attitudes to smoking. There is no objective instrument that will tell us whether smoking is unconsciously felt to be a sin, yet that is one of the conditions for heightened ability to quit or cut down, if one of our theories is correct. There is no objective instrument that will tell us whether smoking is seen as a morally neutral act that has value only as "okay behavior" in the peer group, yet that has been postulated to be the explanation for people who smoke early and "plateau" at a relatively moderate smoking rate. Our theory, on indirect evidence, is that individual differences in tolerance for smoking have these sorts of psychodynamic explanation.

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A good interview would provide the associational material from which these unconscious meanings of smoking that we postulate could be inferred or their absence established.

The obtaining of certain facts about smoking that are critical to our present formulations would be a good entree into the topic for the interviewer. We need, for instance, memories of first cigarettes. It is our theory that the circumstances of first smoking are very determinative of later behavior. We need to know what the parents said and did about smoking; in many instances the parental attitude must have been, "Do as I say, don't do as I do" or else the inferences we take from our indirect data are all wrong. In short, some pretty obvious opening questions about smoking are available for the interviewer that should, indeed, serve to set the associational chain going, but that should also in their own right contribute validation or infirmation to our existing theories.

The matter of reliability is one that always comes up when we present our preliminary findings for scientific discussion. Other scientists immediately say either, "That instrument is not reliable" or "How do you know that that relationship is reliable?" The first objection arises, in particular, over the Rorschach we have in the Study data. Since this is an abbreviated test that could be repeated in ten minutes, one thinks that the interviewer might collect a good number of retests, so that the reliability of the instrument (over 15 years) could be established. The second argument could be met with regard to several of our personality variables. If the interview was well done (as it must be, to make this program worthwhile), we would have a new and up to date and reasonably valid estimate of several personality factors that have been shown to be related to smoking. This is especially important for any variable that we now claim predicts smoking behavior. If we say to our fellow scientists, "The fact that a man was of Type B when he was 20 predicts that he will smoke heavily by the time he is 35," they at once and universally reply, "But what does that show? Maybe he became a Type C at age 34 and that's why he's smoking heavily now!" There is now way to answer that argument except to go and look at the men now.

Obviously, this larger program would benefit the Study. It would give us invaluable follow-up material; the entire hour will not be devoted to smoking, if only because we have not told our participants of our interest in this topic. It will provide a doctor who is primarily interested in the Study and who will serve as a living focus for it, not to mention his being available to talk to the participants when they drop in on us, as they frequently do. Nor are we uninterested in the way that one good associational hour could provide the dynamic evidence to fuse a record into integrated explanability. We would like to set up predictions before each interview, forming psychodynamic hypothesses about the individual that would either be confirmed or revised by the interview experience, so that our understanding of each person would be solidified or advanced. Our knowledge of the dynamics would improve our knowledge of the dynamics of his smoking. And some of these advance hypotheses would certainly relate to his smoking behavior.

The tangible product of this work might be several books. One should relate to the psychodynamics of smoking, in some clinical richness, with quotations and case histories spelling out in some detail what is now inferred quite indirectly from fragments of a datum here and a datum there. Nor would our present theories be likely to remain unchanged and unimproved after exposure to so much good cross-validation.

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6. Budget Plan:

Salaries (including Retiring Allowances	$59,200
Expendable Supplies	1,200
Permanent Equipment	1,000
Overhead	5,600
Other TRAVEL EXPENSE	8,000
Total	$75,000

7. Anticipated Duration of Work: Until June 30, 1960

8. Facilities and Staff Available: See Item 1. Presumably one or more of the research assistants who have been active in the project from the outset could be retained.

9. Additional Requirements:

NONE

10. Additional Information (Including relation of work to other projects and other sources of supply):

No other support is available for this purpose.

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Signature /S/ DANA L. FARNSWORTH
Director of Project

/S/ JAMES R. REYNOLDS
Business Officer of the Institution

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#152R1

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

(Originally Activated 7/1/57)

Application For Research Grant

Date: April 4, 1958

1. Name of Investigator: Charles B. McCants Ph.D.

2. Title: Arsenic content of soils and absorption by the tobacco plant

3. Institution & Address: Department of Soils
North Carolina State College
Raleigh, North Carolina

4. Project or Subject:

Since cancerous conditions have been developed through the use of arsenic, the deduction has been made that arsenic inhaled in cigarette smoke is a contributing factor in the development of cancer. It has been further hypothesized that tobacco absorbs appreciable quantities of arsenic from the soil. The objectives of this study are:

1. To determine the arsenic content and distribution in soils used in the production of flue-cured tobacco.
2. To study the arsenic absorption by tobacco from soils as it is influenced by soil type, by the arsenic content of the soil and by fertility practices.
3. To study the effect of certain soil properties on the retention of arsenic in the soil.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The experimental procedure will be, in general, a continuation and amplification of that outlined in "Application for Research Grant", February 20, 1957.

Field experiments were initiated in 1957 on three soil types typical of those used in flue-cured tobacco production. In each experiment there are seven variables with respect to applied arsenic. The experiments will be continued on the same sites in 1958, but no additional arsenic will be applied to the soil and none used on the plants. To each of the arsenic variables will be applied two rates of phosphorus in 1958. Soil and plant samples will be taken periodically during the year from each treatment and from all locations. Data from these samples will provide information on the residual effects of arsenic applied in 1957 and on the influence of phosphorus on the distribution of arsenic in the soil and absorption of arsenic by the plant.

The survey initiated in 1957 will be continued on a more extensive basis in 1958. Only sites on which arsenic has not been applied to the current crop will be included in the survey. Data from the soil and plant samples obtained in the survey will aid in determining the residual arsenic levels in soils used for flue-cured tobacco production and in estimating the magnitude of arsenic absorption from the soil.

Work initiated in 1957 on methods for analyzing soils and plants for arsenic will be intensified. Preliminary investigations have indicated the need for a method that is more reliable and reproducible than is now available and one that is adaptable to the large number of samples that are to be analyzed. When satisfactory methods have been achieved, the soil and plant samples collected in 1957 and 1958 will be analyzed.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

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6. Budget Plan:

Salaries	$2,500
Expendable Supplies	300
Permanent Equipment	200
Overhead	
Other	
Total	$3,000

7. Anticipated Duration of Work: Two years

8. Facilities and Staff Available:

Soils Department, North Carolina State College

C. B. McCants, Leader
W. G. Woltz, Cooperator
W. V. Bartholomew, Cooperator
N. T. Coleman, Cooperator
J. W. Fitts, Cooperator

9. Additional Requirements:

A graduate student has been assigned to the project. This student has been assisting, on a part time basis, in the work on this project since its initiation and hence is familar with the objectives and procedures. Additional labor will be furnished to assist in the field and laboratory work. Routine analyses will be made in the Departmental Service Laboratory.

10. Additional Information (Including relation of work to other projects and other sources of supply):

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Signature J. W. Fitts
~~Director of Project~~ Head, Soils Dept.

[illegible] Director of Research

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant #152

Date: February 20, 1957

1. Name of Investigator: Charles B. McCants, Department of Soils

2. Title: Arsenic content of soils and absorption by the tobacco plant

3. Institution & Address: North Carolina State College, Soils Department, Raleigh, North Carolina

4. Project or Subject: Since cancerous conditions have been developed through the use of arsenic, the deduction is made that the arsenic inhaled in cigarette smoke is a contributing factor toward the development of cancer.

1. To determine the arsenic content and distribution in soils used for the production of flue-cured tobacco.

2. To study the arsenic absorption by the tobacco plant from soils as influenced by soil type, by the arsenic content of the soil and by fertility practices.

3. To study the effect of certain soil properties on the retention of arsenic in the soil.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Representative surface and subsoil samples will be obtained from the major flue-cured tobacco areas in North Carolina. The random selection of the sampling sites will be modified where necessary to include the predominate soil types used in the production of tobacco. Included in these samples will be those from fields on experiment stations and farms from which can be obtained samples of field-grown tobacco that have received no arsenical sprays or dusts.

The soil samples will be analyzed for extractable arsenic, phosphorus, and such other chemical properties as may seem appropriate. The samples will represent various depths for a given soil in order to locate the area of highest arsenic concentration. When the analysis indicates an abnormally high level of arsenic in a soil, efforts will be made to obtain samples of plants grown on these soils if such plant material is not already available. The per cent arsenic in the plant material will be determined and correlated with the arsenic content of the soil.

As a supplement to the survey phase of the study, field experiments will be initiated in which different amounts of arsenic will be applied to the soil. Arsenic will be applied in only one year, but the experiments will be continued on the same sites for three or more years during which tobacco will be the test plant. The arsenic content and distribution in the soil and arsenic uptake by the plant will be determined for each year. Data from these experiments will provide additional evidence on the absorption of arsenic at various levels of soil arsenic and on changes in available arsenic in the soil with time.

Laboratory, greenhouse and field investigations will be used to study the factors which influence the movement of arsenic in soils. Among the factors to be considered will be the soil type, the phosphorus concentration in the soil and phosphorus applications.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	$2,500
Expendable Supplies	300
Permanent Equipment	200
Overhead	
Other	
Total	$3,000

7. Anticipated Duration of Work: Three years.

8. Facilities and Staff Available:

Soils Department, North Carolina State College
C. B. McCants, Leader
W. G. Woltz, Cooperator
W. V. Bartholomew, Cooperator
M. T. Coleman, Cooperator
J. W. Fitts, Cooperator

9. Additional Requirements:

One graduate student with a M.S. degree working towards a Ph.D. degree will be assigned to this project. Additional hourly labor will be furnished for field and laboratory work. Routine analyses will be made in the Departmental Service Laboratory.

10. Additional Information (Including relation of work to other projects and other sources of supply):

100354158

Signature /s/ J. W. Fitts Charles B. McCants
Director of Project

/s/ Dr. R. L. Lovvorn
Business Officer of the Institution
Director of Research
N. C. Agricultural Experiment Station

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Tob. Science Vol. 168, No. 19 May 8, 1959

Arsenic Content of Commercial Tobacco, 1917-1958[1]

F. E. Guthrie, C. B. McCants and H. G. Small, Jr.

Departments of Entomology and Soils
North Carolina State College, Raleigh, North Carolina, U.S.A.

Arsenic in tobacco was first reported by Remington (1927), and since then this subject has been reviewed in a number of publications. Recently, Satterlee (1956) reported that the arsenic content of cigarettes increased steadily from 1932 to 1951 and hypothesized that cigarette tobacco would continue to contain large amounts of arsenic due to absorption from the soil.

The presence of naturally occurring arsenic in plants and foods has been reported a number of times and is especially high in certain edible marine life (Coulson *et al.* 1934).

Since 1952, arsenic compounds have been removed from the list of recommended insecticides for control of tobacco hornworms, and strenuous efforts have been made to reduce the use of these materials on tobacco. These efforts have been successful as indicated by the work of Bailey *et al.* (1956) and Weber (1956) who showed that the arsenic content of tobacco has declined sharply since 1952.

Widespread publicity has been given to a recent paper by Holland *et al.* (1958) in which cigarettes purchased in 1957 reportedly contained 42 to 52 ppm. of arsenic and in which speculations were made on the health aspects of such high levels of arsenic in cigarettes. In view of the interest in this subject, data are presented on the arsenic content of cigarettes and tobacco samples obtained during the period 1917-1958 and analyzed at this institution.

Methods and Materials

Two state agencies, working independently of each other, have analyzed a number of tobacco samples collected during the period 1917-1958. The paper from each 20 cigarette sample was removed prior to oven-drying. The composite samples of unmanufactured tobacco consisted of at least 50 leaves drawn at random from larger lots of tobacco. The oven-dried samples (65°C.) were ground in a Wiley mill through a 40 mesh screen. One gram samples were used for each analysis, and the average of duplicate samples was taken. Both laboratories used a wet digestion method for extracting arsenic. Arsenic in the digestate was determined by the Gutzeit method in the Chemistry Division, N. C. Department of Agriculture[2] and by the molybdenum-blue colorimetric test in the Soils Department, N. C. State College. (The specific details of the latter method will be described at a later date.)

The tobacco samples were obtained from the following sources:

1917, 1931, 1935, 1951—commercial brands of cigarettes.
1953—composite sample of tobacco from the Middle Belt.

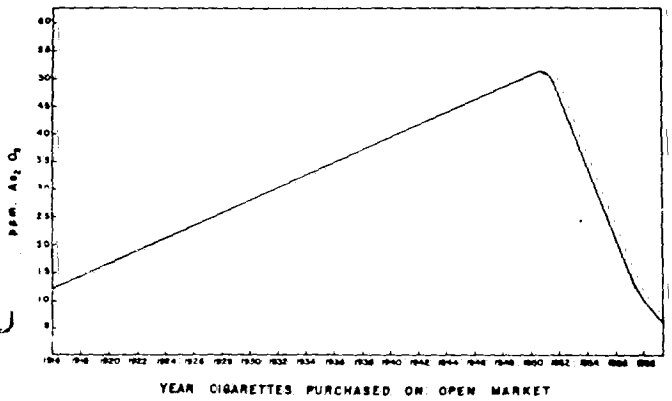

Figure 1. Arsenic content of commercial cigarettes, 1917-1958.

[1] *Contribution from the Entomology and Soils Departments, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 1005 of the Journal Series.*

[2] *The authors express appreciation to Mr. J. G. Jernigan of the Chemistry Division, N. C. Department of Agriculture, Raleigh, for analysis of the samples by the Gutzeit method.*

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

1954—composite of samples taken from the Flue-Cured Tobacco Stabilization Corporation Warehouse in Raleigh, N. C. and representing tobacco from the Border, Eastern and Middle Belts.

1954—composite sample of tobacco from the Eastern Belt.

1954—composite sample of tobacco from the Middle Belt.

1954—composite sample of tobacco from the Old Belt.

1955—composite sample of tobacco from the Middle Belt.

1955—composite sample of tobacco from the Old Belt.

1955—composite sample of tobacco from the Eastern Belt.

1956—composite of samples taken from Flue-Cured Tobacco Stabilization Corporation Warehouse in Raleigh, N.C. and representing tobacco from the Border, Eastern and Middle Belts.

1957—composite sample from 50 random piles of tobacco from two warehouses at Chadbourn, N. C.

1957—composite sample from 50 random piles of tobacco from three warehouses at Kinston, N. C.

1957—composite sample from 50 random piles of tobacco from five warehouses at Wilson, N. C.

1957—composite sample from 50 random piles of tobacco from one warehouse at Reidsville, N. C.

1957—three popular brands purchased in February.

1958—six popular brands purchased in April and an additional 11 popular brands purchased in December.

Table 1.—Arsenic content of commercial cigarettes, 1917-1958

Sample Number	Brand	Type	Year	ppm., As_2O_3 Laboratory A*	Laboratory B**
1	A	Regular	1917	12.8	17.5
2	A	"	1931	27.5	26.0
3	A	"	1935	22.0	31.0
4	A	"	1951	56.5	51.0
5	A	"	1957	13.0	
6	D	"	"	12.3	
7	C	"	"	8.5	
Average of three brands sampled in 1957				11.3	
8	B	Regular	April, 1958		5.6
9	E	"	"		5.3
10	C	"	"		7.7
11	D	"	"		11.3
12	F	Filter	April, 1958		10.2
13	G	"	"		14.4
14	H	Regular	December, 1958		2.7
15	A	"	"		6.1
16	I	King	December, 1958		4.8
17	J	Filter	December, 1958		4.1
18	K	"	"		2.8
19	L	"	"		4.5
20	M	"	"		5.2
21	F	"	"		3.5
22	N	Menthol	December, 1958		6.5
23	O	"	"		6.0
24	F	"	"		3.4
Average of 17 brands sampled in 1958					6.2

* Chemistry Division, N. C. Department of Agriculture.
** Soils Department, N. C. State College.

Results and Discussion

The data on arsenic in various commercial cigarettes sampled from 1917 through 1958 are shown in **Table 1** and presented graphically in **Figure 1.**

These data show an increase in arsenic in cigarettes from 1917 to 1951 with a sharp decrease thereafter. These results are in very good agreement with those of Weber (1956). The low levels of arsenic in the cigarettes sampled in 1957 and 1958 are also in agreement with the results obtained on samples of unmanufactured tobacco as shown by the data in **Table 2.** These data show further that the two laboratories obtained similar results on the concentration of arsenic in the unmanufactured tobacco. The arsenic levels on commercial cigarettes purchased in 1958 are seven to eight times lower than the 52 ppm. reported by Holland *et al.* (1958).

Both Satterlee (1956) and Shaw (1956) have suggested that many of the soils used in tobacco production contain large amounts of arsenic as residues from previous applications of arsenicals to crops and that tobacco grown on these soils will absorb appreciable amounts of arsenic. Weber (1956) concluded that there was no appreciable absorption of arsenic by tobacco grown on soils in which previous crops had been treat-

Table 2.—Arsenic content of flue-cured tobacco from various sources, 1953-1957.

Year	Source	ppm., As_2O_3 Laboratory A*	Laboratory B**
1953	Middle Belt	8.0	10.0
1954	Gov. Storage	0.1	1.4
1954	Eastern Belt	5.8	5.4
1954	Middle Belt	7.5	7.8
1954	Old Belt	6.8	5.8
1955	Eastern Belt	5.3	3.5
1955	Middle Belt	5.5	4.4
1955	Old Belt	7.0	4.2
1956	Gov. Storage	7.3	3.5
1957	Chadbourn	1.8	1.8
1957	Wilson	10.8	8.2
1957	Kinston	0.8	2.0
1957	Reidsville	4.0	2.5

* Chemistry Division, N. C. Department of Agriculture.
** Soils Department, N. C. State College.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

ed over a long period of time with arsenic. A study on the absorption of arsenic by tobacco grown in soils containing different levels of arsenic is presently being conducted by the Soils Department, N. C. State College.

Summary

Data are presented on the arsenic residues found on samples of tobacco taken from 1917-1958. The peak years for arsenic content in tobacco were during the early 1950's, and since then there has been a continued decrease. The evidence here is that the arsenic content of present-day American cigarettes is within the range of 3-12 ppm. with an average of approximately 6 ppm. These data, plus the fact that the usage of arsenic on tobacco has been practically nil since 1952, make it difficult to rationalize any increase in arsenic in cigarettes since 1952 or the presence of the reportedly high levels of arsenic in cigarettes at the present time.

Literature Cited

Bailey, E. J., E. L. Kennaway and M. E. Urquhart. Arsenic content of cigarettes. *Brit. Jour. Cancer* 11: 49-53 (1956).

Coulson, E. J., R. R. Remington and K. M. Lynch. Toxicity of naturally occurring arsenic in foods. *Science* 80: 230-231 (1934).

Holland, R. H., R. H. Wilson, A. R. Acevedo, M. S. McCall, D. A. Clark and H. C. Lanz. A study of arsenic in regular-sized unfiltered and filtered cigarettes. *Cancer* 11: 1115-1118 (1958).

Remington, R. E. A hitherto unsuspected source of arsenic in human environment. *Jour. Amer. Chem. Soc.* 49: 1410-1416 (1927).

Satterlee, H. S. The problem of arsenic in American cigarette tobacco. The *New England Journal of Medicine*. 254: 1149-1154 (1956).

Shaw, R. R. (Press release at Conference of the American Cancer Society, October 5, 1956, San Francisco) (1956).

Weber, J. H. Arsenic in cigarette tobacco. *Jour. Sci. Food Agr.* 8: 490-1 (1956).

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

TIRC Grant #152, -R1

CONFIDENTIAL

Charles B. McCants, Ph.D.
North Carolina State College

Progress Report #2
May 7, 1959

"Arsenic Content of Soils and Absorption by the Tobacco Plant."

Field experiments in which seven rates of arsenic were applied to three different soil types in 1957 were continued on the same sites in 1958 with no further application of arsenic. These experiments were designed to obtain data on the absorption of residual arsenic from the soil and on the distribution of arsenic in the soil. A phosphorus variable was included in 1958 on all treatments and at all locations to study the influence of phosphorus on arsenic absorption.

In the 1958 experiments, the sampling procedures were similar to those employed in 1957. Soil samples were obtained at three depths in all treatments and locations and samples of cured tobacco obtained from all plots.

An extensive sampling of farmer-grown tobacco and fields of known management and cropping history was conducted.

Investigations were continued on methods for the analyses of soils and plants for arsenic. A method for plant analysis has been developed which gives good apparent recovery of arsenic, excellent reproducibility and is adaptable to routine analyses. This method has been employed in the analysis of certain of the samples obtained in 1957. Results of these analyses are given in Table 1.

While the analyses of plant samples from the 1958 experiments and survey have not been completed, the data available at this time show a close similarity to those from the 1957 experiments. The data do not suggest any direct effect of phosphorus on the absorption of arsenic.

Analyses of samples of farmer-grown tobacco on which no arsenical insecticides were applied show that the level of arsenic in the leaf is similar to that found in experiments where no arsenic was applied to the soil.

Since a satisfactory procedure for analysis of soils for arsenic is not yet available, no data are yet available on the arsenic distribution in soils. It is anticipated that the procedure now being developed will be completed shortly and that analysis of the soil samples will commence within three months.

Numerous samples of commercial cigarettes and cigarette tobaccos have been analyzed for arsenic for both college and industry representatives. The enclosed paper was prepared from data obtained largely by procedures developed under TIRC Grant 152 and by personnel working under this grant. This paper is being made a part of this report.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Table 1. Arsenic content of cured tobacco from three field experiments in which variable rates of arsenic were applied to the soil. 1957

Arsenic applied to soil 1/ lb. As_2O_3/A	Arsenic in leaf, ppm As_2O_3		
	Whiteville 2/	Clayton 3/	Rural Hall 4/
0	1.9	1.4	0.8
2	3.3	2.3	1.5
4	3.5	3.1	1.4
8	4.2	4.5	1.1
16	7.4	8.5	0.9
32	7.3	11.4	1.9
64	9.6	7.8	3.3

1/ When arsenicals were used as insecticides, the rate was equivalent to approximately 2 lb. As_2O_3 per acre per year.
2/ Soil type: Goldsboro fine sandy loam
3/ Soil type: Norfolk sandy loam
4/ Soil type: Cecil sandy clay loam

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#152R2

TOBACCO INDUSTRY RESEARCH COMMITTEE

Activated 7/1/57
Renewed 7/1/58

150 East Forty Second Street New York 17, N.Y.

Date: May 7, 1959

1. Name of Investigator: Charles B. McCants

2. Title: Arsenic content of soils and absorption by the tobacco plant.

3. Institution & Address: Department of Soils
North Carolina State College
Raleigh, North Carolina

4. Project or Subject: Since cancerous conditions have been induced through the use of arsenic, the deduction has been made that arsenic inhaled in cigarette smoke is a contributing factor in the development of cancer. It has been further hypothesized that tobacco absorbs appreciable quantities of arsenic from the soil. The objectives of this study are:
1. To determine the arsenic content and distribution in soils used in the production of flue-cured tobacco.
2. To study the arsenic absorption by tobacco from soils as it is influenced by soil type, by the arsenic content of the soil and by fertility practices.
3. To study the effect of certain soil properties on the retention of arsenic in the soil.

5. Detailed Plan of Procedure
The nature of this research requires experimentation with field-grown tobacco and, therefore, at least three years investigations are necessary. The experimental procedures are, in general, an amplification of those described in applications of February 20, 1957 and April 4, 1958.

The field experiments initiated in 1957 and continued in 1958 consisted of seven rates of arsenic applied to each of three soils. These experiments will be continued with no further application of arsenic to study the absorption of residual arsenic from the soil and the distribution of arsenic in the soil. From the various treatments, leaves of cured tobacco will be obtained from several stalk positions to determine the distribution of arsenic in the plant as well as the arsenic content of the lamina and stem portions of the leaves. In addition to the field experiments, greenhouse and laboratory experiments will be conducted to study the absorption of arsenic through leaves compared to absorption by roots.

The survey initiated in 1957 and expanded in 1958 will be further increased in 1959 to study the status of arsenic in certain North Carolina soils and the arsenic content of farmer-grown tobacco.

Additional work is necessary to develop a procedure for analysis of soils for arsenic, since published methods have not proven satisfactory. Progress is being made on a procedure and when additional refinements have been perfected, soil samples from the 1957 and 1958 experiments will be analyzed.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Since advocates of the arsenic-cancer relationship have intimated that wet digestion procedures for arsenic analysis of plants generally give lower recovery of arsenic than does the bomb-combustion method, a comparative study of the accuracy of the two methods will be made. Should the results of this study indicate a significant discrepancy, appropriate modifications will be made in the procedure for plant analysis to enable the maximum recovery of arsenic in tobacco.

6. Budget Plan:

Salaries	$2,500.
Expendable Supplies	300.
Permanent Equipment	200.
Overhead	
Other	
Total	$3,000.

7. Anticipated Duration of Work: One year

8. Facilities and Staff Available: Soils Department, North Carolina State College

C. B. McCants, Leader
W. G. Woltz, Cooperator
W. V. Bartholomew, Cooperator
N. T. Coleman, Cooperator
J. W. Fitts, Cooperator

9. Additional Requirements:

A graduate student, Mr. Howard G. Small, Jr., has been assigned to the project. This student has been assisting in various capacities in the work on this project since its initiation and hence is familiar with the objectives and procedures. Additional labor will be furnished to assist in the field and laboratory work. Routine analyses will be made in the Departmental Service Laboratory.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Signature /s/ J. W. Fitts
Head, Soils Dept.

/s/ Kenneth R. Keller
Asst. Director of Research

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#152R2

TOBACCO INDUSTRY RESEARCH COMMITTEE

Activated 7/1/57
Renewed 7/1/58

150 East Forty Second Street New York 17, N.Y.

Date: May 7, 1959

1. Name of Investigator: Charles B. McCants

2. Title: Arsenic content of soils and absorption by the tobacco plant.

3. Institution & Address: Department of Soils
North Carolina State College
Raleigh, North Carolina

4. Project or Subject: Since cancerous conditions have been induced through the use of arsenic, the deduction has been made that arsenic inhaled in cigarette smoke is a contributing factor in the development of cancer. It has been further hypothesized that tobacco absorbs appreciable quantities of arsenic from the soil. The objectives of this study are:
1. To determine the arsenic content and distribution in soils used in the production of flue-cured tobacco.
2. To study the arsenic absorption by tobacco from soils as it is influenced by soil type, by the arsenic content of the soil and by fertility practices.
3. To study the effect of certain soil properties on the retention of arsenic in the soil.

5. Detailed Plan of Procedure
The nature of this research requires experimentation with field-grown tobacco and, therefore, at least three years investigations are necessary. The experimental procedures are, in general, an amplification of those described in applications of February 20, 1957 and April 4, 1958.

The field experiments initiated in 1957 and continued in 1958 consisted of seven rates of arsenic applied to each of three soils. These experiments will be continued with no further application of arsenic to study the absorption of residual arsenic from the soil and the distribution of arsenic in the soil. From the various treatments, leaves of cured tobacco will be obtained from several stalk positions to determine the distribution of arsenic in the plant as well as the arsenic content of the lamina and stem portions of the leaves. In addition to the field experiments, greenhouse and laboratory experiments will be conducted to study the absorption of arsenic through leaves compared to absorption by roots.

The survey initiated in 1957 and expanded in 1958 will be further increased in 1959 to study the status of arsenic in certain North Carolina soils and the arsenic content of farmer-grown tobacco.

Additional work is necessary to develop a procedure for analysis of soils for arsenic, since published methods have not proven satisfactory. Progress is being made on a procedure and when additional refinements have been perfected, soil samples from the 1957 and 1958 experiments will be analyzed.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Since advocates of the arsenic-cancer relationship have intimated that wet digestion procedures for arsenic analysis of plants generally give lower recovery of arsenic than does the bomb-combustion method, a comparative study of the accuracy of the two methods will be made. Should the results of this study indicate a significant discrepancy, appropriate modifications will be made in the procedure for plant analysis to enable the maximum recovery of arsenic in tobacco.

6. Budget Plan:

Salaries	$2,500.
Expendable Supplies	300.
Permanent Equipment	200.
Overhead	
Other	
Total	$3,000.

7. Anticipated Duration of Work: One year

8. Facilities and Staff Available: Soils Department, North Carolina State College

C. B. McCants, Leader
W. G. Woltz, Cooperator
W. V. Bartholomew, Cooperator
N. T. Coleman, Cooperator
J. W. Fitts, Cooperator

9. Additional Requirements:

A graduate student, Mr. Howard G. Small, Jr., has been assigned to the project. This student has been assisting in various capacities in the work on this project since its initiation and hence is familiar with the objectives and procedures. Additional labor will be furnished to assist in the field and laboratory work. Routine analyses will be made in the Departmental Service Laboratory.

10. Additional Information (Including relation of work to other projects and other sources of supply):

1003541590

Signature /s/ J. W. Fitts
Head, Soils Dept.

/s/ Kenneth R. Keller
Asst. Director of Research

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET, NEW YORK 17, N.Y.

#144

Application For Research Grant

Date: November 13, 1956

1. Name of Investigator: Donald F. McDonald, M.D.
Kenneth P. Knudtson, M.D.
T. Lloyd Fletcher, Ph.D.

2. Title: Experimental Carcinogenicity of Tobacco Smoke Condensate Extractives to Transitional Epithelium of the Urinary Bladder.

3. Institution & Address: University of Washington School of Medicine
Seattle 5, Washington

4. Project or Subject:

The purposes of the project are:

a. To ascertain whether tobacco tar (smoke condensate) extractives are capable of inducing transitional cell tumors of urinary bladder epithelium in mice, rats, and dogs.

b. To identify the substance or substances responsible for a positive result in (a).

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

a. Preparation of smoke condensate extractives (Ecusta Paper Company).

The general scheme of partitioning the smoke condensate into convenient fractions for preliminary testing is as follows:

Tar in suitable solvent

Extract with base - neutralize extract with solvent, dry and evaporate (a)

Residue
Extract with acid - neutralize extract with solvent, dry and evaporate (b)

Residue
Evaporate solvent (c)

(a), (b), (c) will be individually subjected to chromatographic separation, thus, providing $a_{1,2,3}$, $b_{1,2,3}$, $c_{1,2,3}$ and residues.

The objective of preliminary fractionation will be to provide a convenient number of fractions for testing. When testing has been completed, the direction of efforts to identify whatever carcinogen may be found present might be localized to one fraction. Identification of the carcinogen could then be attempted and biologically proved. Because the yield of material in each fraction can be estimated only very roughly, the estimate of amount of

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smoke condensate required cannot be very realistic. Dr. Knudtson is planning to use some of the fractions for a pilot series of intrapulmonic implants to test carcinogenicity of the material to the lung. Pure nicotine will also be tested.

b. Testing of smoke condensate extractives for carcinogenicity.

Because of species differences, we feel that conclusive evaluation of carcinogenicity is best achieved by working with as many species as is practical. For statistically sound results, twenty observations for each substance should be made. We have been working in the field of bladder cancer for seven years. During the past five years, we have been establishing reliable methods for testing carcinogenicity. We are currently testing human urinary solid extractives and residues from patients with urinary bladder tumors, patients with tumors of other organs and normal patients. Dr. Knudtson has helped us in the histological evaluation of some of the material. He has volunteered to help us with histological studies in the present experiment. Because of our experience, we feel we are in an advantageous position to conduct an expeditious, reliable and conclusive evaluation of the carcinogenicity of smoke condensate extractives. The details of methods in the various species follows:

(1) Mice:

Surgical implantation of a pellet of the material incorporated in cholesterol or paraffin can be regarded as a completely satisfactory method of evaluation in this species. Forty to 52 weeks should be a satisfactory induction. Ref.: Bonser, Clayson, Jull and Pyrah, Brit. J. Urol. 26:49-52, 1954.

(2) Rats:

The formation of papillary bladder tumors in rats frequently follows the prolonged stimulus of a urinary calculus. For this reason, Reffo's work cannot be considered reliable. Calculi form about niduses introduced into rat bladders unless they are smooth (e.g., glass). We have found that subcutaneous transplantation of half of the rat bladder provides a bladder pouch, not in contact with the urine, which serves as a very suitable test object for testing of topically effective carcinogens. Tumors can form in as little as six months, however, optimum results are obtained in one year.

(3) Dogs:

By surgical partial obstruction of one ureter, a large dilated ureter, which subsequently can be transplanted to a subcutaneous position, provides room for implantation of up to ten different substances. Colored nylon ligatures permit positive identification of the isolated segments of ureter up to two years later. Bladder mucosa can be stripped, wrapped around a polyethylene splint and free-grafted to the subcutaneous tissues of the chest wall. This provides an additional number of testing pouches. Testing in dogs requires eighteen months.

1003541592

6. Budget Plan: For year, 1/1/57 to 12/31/57

Salaries:

Ph.D. Chemist, part-time, 6 mo.	$1,800.00	
Technical Assistant, surgery, histology	3,600.00	$5,400.00

Expendable supplies:

	Initial cost	Food and care		
200 mice	$100.00	$ 50.00		
200 rats	200.00	100.00		
10 dogs	120.00	1,100.00		
			$1,670.00	
Smoke condensate (100 ampoules)			430.00	
Solvents, reagents, glassware			600.00	
Histological supplies			250.00	
Photography			100.00	3,050.00

Equipment: None

SUBTOTAL	$ 8,450.00
Overhead (15%):	1,267.00
	$ 9,717.00

7. Anticipated Duration of Work:

It will probably require six months to prepare the smoke condensate and implant the extractive fractions into the animals. An additional year induction time and six months for preparation, histological evaluation, and summation. During the second year, the expenses will be largely those incident to animal care and feeding, post-mortem and histological preparation and photography. Estimated requirement for the second year is:

Salaries, $3,600.00; animal maintenance, $500.00; supplies, $200.00 -- subtotal, $4,300.00 / 15% overhead of $645.00 -- Totaling $4,945.00.

8. Facilities and Staff Available:

Facilities -- University of Washington Department of Surgery Experimental Animal Surgery.

-- Animal quarters with complete facilities.

-- 14' x 17' chemical laboratory with all facilities, 6' hood, Gramatic balance, #1 International centrifuge, one circular Precision Warburg apparatus with Summerson manometers and vessels, Evelyn colorimeter, Beckman Model B Spectrophotometer.

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--10' x 12' laboratory with facilities for animal handling, post mortem examination, small animal operating, including instruments and sterilizer.

--15' x 12' isotope laboratory with high speed hood, autoscaler and scintillating proportional counters, balance centrifuge, constant temperature bath, hot plates, usual and special glassware.

--12' x 12' office with slide files, binocular research microscope and light.

Staff:

Donald F. McDonald, M.D., Associate Professor of Surgery and Head of the Division of Urology, University of Washington School of Medicine.

K. P. Knudtson, M.D., Assistant Chief, Laboratory Service, U. S. Veterans Administration Hospital, Seattle, Washington.

T. Lloyd Fletcher, Ph.D., Research Associate Professor, Director of the Department of Surgery Chemistry Research Laboratory, University of Washington School of Medicine, Seattle, Washington.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Enclosed are reprints of previous work done in closely related fields, entitled:

"The Role of the Urine in Vesical Neoplasm. I. Experimental Confirmation of the Urogenous Theory of Pathogenesis."

"Clinical Implications of Transplantability of Induced Bladder Tumors to Intact Transitional Epithelium in Dogs."

Because our studies of carcinogenicity of human urine solids have not been completed (we still have 22 dogs to finish), no published material is available.

Signature /s/ Donald F. McDonald
Director of Project

/s/ Henry N. Harkins
Executive Officer, Dept. of Surgery

C. N. Smiley
Dean - School of Medicine

/s/ for: Nelson A. Whittum
Comptroller

T0035459

Source: https://www.industrydocuments.ucsf.edu/docs/fxnn0000

Cardiovascular, Pharmacology and chemistry

#174R3

Committee:
Dr. Cattell, Chairman
Dr. Reimann
Dr. Bing
Dr. Lynch

#174 activated 2/1/58
#174R1 activated 2/1/59
#174R2 activated 2/1.60

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty-second Street New York, New York

Application For Research Grant

October 26, 1960

1. Name of Investigator: Henry C. McGill, Jr., M.D.

2. Title: Professor and Head, Department of Pathology

3. Institution & Address: Louisiana State University - School of Medicine
1542 Tulane Avenue
New Orleans 12, Louisiana

4. Project or Subject: Relationship of Smoking, Alcohol, and Physical Activity to Coronary Atherosclerotic Lesions.

1003541595

5. Detailed Plan of Procedure:

PURPOSE - The purpose of this study is to determine the relationship between coronary artery atherosclerotic lesions and certain lifetime habits.

MATERIALS AND METHODS - The study cases will include 200 white and Negro males and females, ages 20 to 69, autopsied at Charity Hospital and Office of the Coroner, Orleans Parish. Approximately half of these will be accidental and traumatic deaths, which represent the best possible sample of the living population.

At autopsy the coronary arteries are dissected from the heart, opened and flattened on cardboard, fixed in formalin, stained grossly with a fat stain and sealed in plastic bags for examination and storage. The extent of fatty streaks, fibrous plaques, complicated lesions and calcified plaques in each of the major branches of the coronary arteries will be graded by visual estimation and by electronic flying spot image analysis of photographic and soft tissue X-ray transparencies of the specimens.

Smoking habits, alcohol intake and physical activity of each autopsied case in the study will be investigated by interviewing the closest survivor with a standard questionnaire which has been designed with the assistance of a biostatistician and other experts in questionnaire design. The questionnaire is now being checked on living subjects and their families to test the validity of recall of a family member as to habits of another member. Other tests of the validity of this type of recall will be made during the definitive study. Each subject will be ranked according to each of a number of different measures of tobacco usage, alcohol intake, and physical activity.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Finally, statistical tests for degree of correlation between each measure of lifetime habits and each measure of coronary artery lesions will be carried out.

6. Budget Plan: (Budget for one year).

Salaries	$ 9,828.00
Expendable Supplies	600.00
Travel for Field Interviewer	800.00
Overhead (15%)	1,864.00
Other (Statistical Services)	1,200.00
Total	$14,292.00

7. Anticipated Duration of Work:

Two years minimum.

8. Facilities and Staff Available:

Facilities include the necropsy services of the L.S.U. Service at Charity Hospital and of the Office of the Coroner, Orleans Parish; a technical staff of four individuals (support for one of which is requested in this application) who collect the anatomic specimens and process them; a biophysicist and his technical assistant, who operated the electronic grading apparatus; and biostatistical services and consultation from Dr. C. A. McMahan of the Biostatistics Division of the Department of Public Health and Preventive Medicine.

9. Additional Requirements:

None

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10. Additional Information:

This study is part of a much larger study of the natural history and epidemiology of atherosclerotic lesions being carried on by this laboratory in cooperation with 11 other pathology laboratories in Central and South America and Africa. All of the equipment and information gained from this larger study will be used to some degree in the execution of this project. Paralleling this study of smoking, alcohol and physical activity in relation to atherosclerotic lesions will be a study of the dietary habits of the same individuals being carried out by the Nutrition Division of the Louisiana State Department of Health. The major portion of this work is being supported by the National Heart Institute of the U. S. Public Health Service in grants to L.S.U. and to the Pan American Sanitary Bureau.

This project will be under the immediate supervision of Dr. Jack P. Strong, Associate Professor of Pathology and Senior Research Fellow of the USPHS.

/s/ Henry C. McGill, Jr., M.D.
Director of Project

/s/ _____
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

rec comment

Application For Research Grant

Date: September 9, 1954

1. Name of Investigator: Kelly T. McKee, M.D.

2. Title: Associate Professor of Medicine

3. Institution & Address: Medical College of South Carolina
Charleston, South Carolina

4. Project or Subject: Study of lung function in smokers and non-smokers.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

We propose to measure vital capacity, maximal breathing capacity, nitrogen washout rate, and air flow in a group of healthy young adults, approximately half of whom will be smokers and half non-smokers. It is expected that data will be accumulated which may be of value in estimating the effects of tobacco smoking upon lung function.

No statement as to the number of subjects to be studied can be made at this time, but we expect to study a group large enough to permit conclusions with regard to the problem stated.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries (technician)	$ 2800.00
Expendable Supplies	500.00
Permanent Equipment	4000.00
Overhead	600.00
Other	
Total	$ 7900.00

7. Anticipated Duration of Work:

One year.

8. Facilities and Staff Available:

Laboratory space at the Medical College of South Carolina
Facilities of Pulmonary Function Laboratory at M.C.S.C.

Laboratory Technician, Research Fellow, Advisory assistance from various Clinical and Pre-clinical departments at M.C.S.C.

9. Additional Requirements:

In addition to the available facilities of our Pulmonary Function Laboratory, we will need certain equipment, including a nitrogen meter and recording devices, and Flowmeter and recording devices. It is this equipment that is to be purchased with funds requested under the heading of permanent equipment above.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The project outlined is different from but akin in that it is study of pulmonary function, to work presently being done by our Pulmonary Function Laboratory. Our work thus far has been related to lung function in disease states.

10003541598

Signature Kelly T. McKee
Director of Project

H. M. Fairley
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Committee:
Cattell
Bing
Wilson

#277

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

Application for Research Grant

Date: May 13, 1960

1. Name of Investigator: Herbert C. McKee, Ph.D.

2. Title: Manager, Industrial Pollution and Analytical Research

3. Institution & Address: Southwest Research Institute
8500 Culebra Road
San Antonio 6, Texas

4. Project or Subject: Changes in Body Chemistry Resulting from Smoking.

5. Detailed Plan of Procedure:

In the development of analytical methods to measure trace constituents of body fluids, it was noticed that one compound appeared in urine and breath samples of smokers, but not in non-smokers. This compound has tentatively been identified as acetonitrile.

In the proposed investigation, additional tests will be conducted to confirm this preliminary observation. Urine samples will be analyzed from approximately 60 subjects, divided into three groups: (1) non-smokers, (2) light to moderate smokers, and (3) heavy smokers. Data will be obtained on all trace constituents which are measured by the present procedures. Statistical analysis of the data will then be performed to determine whether the presence of acetonitrile does correlate with smoking, and to see if a correlation exists between any of the other compounds measured and the smoking habits of the subjects.

Additional details are given in the attached proposal. (Only two copies furnished. Committee members see R.C.H.)

6. Budget Plan:

See proposal for Contractual Information and additional details on cost calculation.

Salaries	2,500.
Expendable Supplies	400.
Permanent Equipment	-
Surcharge	2,500.
Other Services	180.
Total	$5,580.

7. Anticipated Duration of Work: Six months.

8. Facilities and Staff Available: See attached proposal.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

9. Additional Requirements: None

10. Additional Information: See attached proposal.

Signature: Wm. E. Thompson
Director of Project

D. D. Bilts
Business Officer of Institution

1003541600

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Committee:
Dr. Cattell, Chm.
Dr. Bing
Dr. Jacobson

#223R1

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, New York

Activated: 7/1/59
Cf. #128
Activated: 7/1/56
Renewed: 7/1/57
Renewed: 7/1/58

Application for Renewal of Research Grant

Date: February 6, 1960

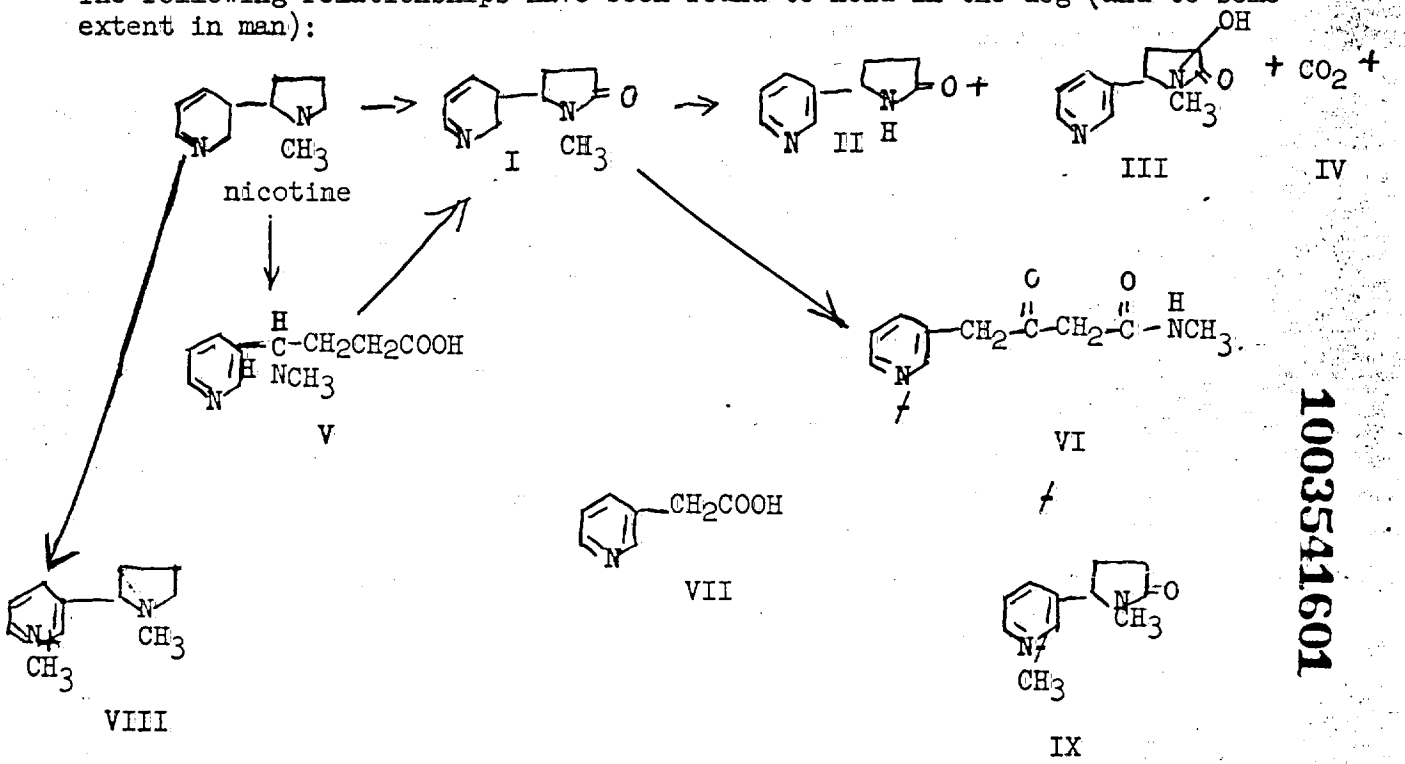
1. Name of Investigator: Dr. Herbert McKennis, Jr. (Dr. Larson)

2. Title: Professor of Pharmacology

3. Institution & Address: Medical College of Virginia
Richmond 19, Virginia

4. Project or Subject: Enzymatic Transformation of Nicotine and Related Compounds.

5. Detailed Plan of Procedure: As a result of previous TIRC grants, procedures have been developed for the isolation and synthesis of metabolites of nicotine. The following relationships have been found to hold in the dog (and to some extent in man):



The studies have thus far indicated that quaternization of nicotine leading to VIII represents a major metabolic route which may account for as much as ten per cent of the dose and the methylation of cotinine (1) to the product IX is important only when large amounts of cotinine is present in the body. Evidence also indicates the probability that additional quaternary ammonium compounds rise during the metabolism of nicotine.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

It becomes desirable to investigate further the quaternary ammonium fraction of urine of nicotine-treated animals in view of the known facility of the body to methylate pyridine rings and the wide spectra of cholinergic and anticholinergic activity which can be anticipated to occur in compounds of this type.

The isolation of 3-pyridylacetic acid (VII) as a result of metabolism of cotinine at once raises the possiblity that 3-pyridylacetic acid arises in vivo as a result of the enzymatic cleavage of the metabolite γ -(3-pyridyl)-β -oxo-N-methylbutyramide (VI). Studies in vitro indicate that the keto acid from VI has an exceptional stability to both acid and base. This points to the necessity for an enzymatic reaction if the foregoing precussor-metabolite relationship obtains in vivo. A study of the metabolism of both VI and VII appear to hold great promise in helping to account for additional factors in the metabolism of nicotine. This is especially true in the case of 3-pyridylacetic acid (VII) since it has been reported (Ratti and de Fini, The Lancet, 2, 917, 1959) that quantities of 3-pyridylacetic acid in the range of 1.5-2.5 grams per day are tolerated by humans.

It is proposed to continue studies on the metabolism of nicotine in accordance with the foregoing with the general objective of creating a sound foundation which will lead to an eventual understanding of the biological significance of the metabolic events.

6. Budget Plan:

Social Security	426.00
Salaries	20,000.00
Expendable Supplies	4,500.00
Permanent Equipment	7,385.00
Overhead (10%)	3,320.00
Other (Travel)	890.00
Total	$36,521.00

7. Anticipated Duration of Work: Two years additional. (First annual renewal of the present grant which expires June 30, 1960). Progress will be reported at intervals and in the literature according to custom and past practice.

8. Facilities and Staff Available: General pharmacologic and biochemical equipment, Lardy type Warburg apparatus, refrigerator centrifuge, C^{14} counting equipment, nuclear instrument glass flow counter with automatic sample changer, paper chromatographic equipment, polarimeter, greenhouse facilities for growing C^{14} labelled plants, high pressure hydrogenation apparatus. Full time staff: Lennox B. Turnbull, Ph.D., M.S. to be hired, Part time includes: Drs. Herbert McKennis, Jr., Paul S. Larson, H. B. Haag, and J. F. Borzelleca, E. R. Bowman, Public Health Research Fellow and S. Schwarz, graduate student.

9. Additional Requirements: What, if any, will depend upon how project develops.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The American Tobacco Company has, as in the past, provided C^{14} nicotine. E. R. Bowman has received a fellowship from the National Heart Institute. S. Schwarz expects to receive a fellowship from the National Science Foundation.

100354602

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Dr. Herbert McKennis, Jr. (Larson) February 6, 1960

The breakdown of salaries is as follows:

Lennox B. Turnbull,	Ph.D.	$9,200.
To be filled	M.S.	6,200.
Technician	B.S.	3,600.
Animal man part-time		1,000.
		$ 20,000.

The breakdown on permanent equipment is as follows:

Microelectrophoresis apparatus	1,555.
Actigraph chromatogram canner (with accessories)	2,700.
Water bath shaker	630.
Spectrophotofluorometer (part of cost)	2,500.
	$ 7,385.

/s/ Herbert McKennis, Jr.
Director of Project

/s/ W. F. Tompkins
Business Officer of the Institution

1003541603

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE (#225 CF #128
Activated 7/1/56
150 East Forty Second Street Renewed 7/1/57
" 7/1/58)
New York 17, N.Y.

Application For Research Grant

Date: December 19, 1958

1. Name of Investigator: Herbert McKennis, Jr., Ph. D.

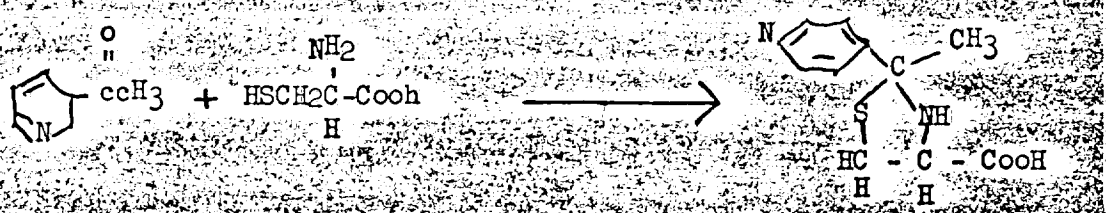
2. Title: Professor of Pharmacology

3. Institution & Address: Medical College of Virginia
Richmond 19, Virginia

4. Project or Subject: Metabolism of Antinicotinic Acid Compounds of Tobacco Smoke

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):
In addition to nicotine tobacco smoke contains a variety of pyridine compounds including cotinine and acetylpyridine (Quinn, Twelfth Tobacco Chemists' Research Conference). Current studies in this laboratory have done much to establish that the nicotine metabolite cotinine is remarkably devoid of gross physiological effects in comparison with the parent compound nicotine. Recent work in other laboratories has tended to implicate nicotinic acid deficiency as a causative factor in experimental cancer. 3-Acetylpyridine already has assumed an important role as an experimental antagonist of nicotinic acid. In addition it has been observed that extensive brain damage arises (Hicks, Am. J. Pathol. 31, 189 (1955); Coggeshall and MacLean, Proc. Soc. Exp. Biol. Med. 98, (1958)). If it is assumed that the deleterious effects arise from 3-acetylpyridine itself or a condensation product in the form of pyridine nucleotides, it is interesting to contemplate the possibility that small amounts of 3-acetylpyridine, such as are present in smoke, are metabolized to compounds which do not in themselves possess the physiological actions of 3-acetylpyridine.

Preliminary experiments (unpublished from this laboratory) have shown that 3-acetylpyridine is metabolized by the dog to a minimum of at least 4 Koenig positive components. These have been separated by chromatographic methods but not in sufficient quantity for chemical identification and physiological studies. Preliminary evidence also indicates that 3-acetylpyridine will react with cysteine to form a thiazolidine:



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It is proposed to administer 3-acetylpyridine and isolate by chromatographic techniques sufficient quantities for structural determinations and subsequent synthesis in quantities sufficient for biochemical studies. Compounds arising from the metabolism of 3-acetylpyridine which do not give the Koenig reaction can by fractionated by means of a C^{14} label (obtained from C^{14} nicotinic acid which is available for the synthesis of 3-acetylpyridine).

6. Budget Plan:

Social Security	$ 240
Salaries	$ 11,000
Expendable Supplies	$ 4,500
Permanent Equipment	$ 3,000
Overhead (10%)	$ 1,939
Other (Travel)	$ 650
Total	$ 21,329

7. Anticipated Duration of Work: Three years.

8. Facilities and Staff Available: General pharmacologic and biochemical equipment, Warburg apparatus, greenhouse for growing C^{14} labelled pyridine compounds, Carey recording spectrophotometer, C^{14} counting equipment.

9. Additional Requirements: What, if any, will depend upon how the product develops.

10. Additional Information (Including relation of work to other projects and other sources of supply): Staff to be hired: Ph.D. full time, $8,000, technician, $3,000. Part-time will include Dr. Herbert McKennis, Jr. and Edward S. Bowman, Public Health Research Fellow. Other personnel with experience in pyridine compounds will be available for consultation as required.

The breakdown on equipment is:

pH Meter	$ 500
Deep Freeze	$ 500
Ice box	$ 400
Part cost of infrared equipment	$ 1,600
	$ 3,000

/s./ Herbert McKennis, Jr.
Director of Project

/s./ B. B. Smith
Business Officer of the Institution

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TOXICOLOGY 1, 61-64 (1959)

A Constant-Rate Infusion Apparatus

QUENTIN S. MCKENNIS, EDWARD R. BOWMAN,[1] AND HERBERT MCKENNIS, JR.[2]

Department of Pharmacology, Medical College of Virginia, Richmond, Virginia

Received August 15, 1958

INTRODUCTION

In a variety of pharmacologic and chemical studies there is a requirement for a device which will permit the infusion of solutions at a constant rate to an organism or chemical mixture. For simple chemical reactions there are a variety of devices for proportionate feed or constant dropwise addition of reagents (Humphlett, 1955). In pharmacologic studies the infusion or constant rate injection of solutions can be accomplished by manual means, which are often tedious and inaccurate, or with the aid of positive-drive mechanisms which are generally elaborate, although, under ideal conditions, trouble-free and accurate. Apparatus in the latter category is usually expensive and, in cases where simultaneous multiple tests are conducted, the cost of the apparatus might well become prohibitive.

In the course of studies in this laboratory (McKennis *et al.*, 1957) where dogs were given aqueous solutions of nicotine intravenously over an 8-hour period it was desirable to develop a moderately priced apparatus for simultaneous infusion at constant rate to six or more animals. By the advantageous selection of inexpensive commercial components, such an apparatus was developed. So far as we are aware, no equivalent device has been described. The wide range of applicability of this simple device prompts a report of it.

EXPERIMENT AND DISCUSSION

Dogs under pentobarbital anesthesia were cannulated at the femoral vein. The cannulas were attached (Fig. 1) by polyethylene tubing to a

[1] Public Health Research Fellow of the National Heart Institute.

[2] The authors are grateful to the American Tobacco Company and the Tobacco Industry Research Committee for their generous support.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

25- or 50-ml burette, equipped with solenoid valve.[3] The burette was enlarged at the top by means of a 60-mm powder funnel which was connected by a short length of tubing to the top of the burette. The solenoid valve was actuated by means of a repeat cycle timer.[4] For the

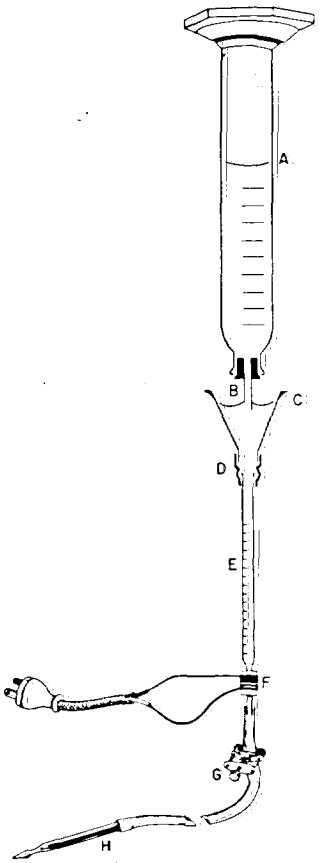

FIG. 1. Schematic drawing of a constant-rate infusion apparatus. *A*, inverted graduated cylinder; *B*, 15-mm o.d. polyethylene tubing fitted by rubber stopper; *C*, powder funnel fitted to burette by tubing; *D*; *E*, 25–50-ml burette with solenoid valve; *F*; *G*, pinch clamp for adjustment of flow to cannula, *H*.

[3] Cat. No. 824-2, Houston Glass Fabricating Company, 5513 Harrison Boulevard, Houston 11, Texas.

[4] Cat. No. 6404, Andrew Technical Supply, 6972 North Clark Street, Chicago 26, Illinois.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

purposes of the study a delivery of 1 ml in each 60-second period was selected. The timer, normally open, was set to close for 1 second in each minute.

Initial adjustment to the required rate of flow is easily achieved by means of the screw clamp (*G*). The burette is filled to the 0.0-ml mark with saline solution or other suitable material. The rate of flow will decrease approximately 0.1 ml per delivery with a hydrostatic head loss corresponding to 1 ml on the burette. In consequence, adjustment of flow rate can be made without the necessity of filling the burette to the zero mark for each delivery during the adjustment period. The desired rate of flow is usually easily achieved with a maximum of five trial deliveries. After this the burette and reservoir systems are filled with the experimental solutions.

TABLE 1

PERFORMANCE DATA ON THE CONSTANT-RATE INFUSION APPARATUS AFTER INITIAL ADJUSTMENT TO DELIVER 1 ML PER MINUTE

Observation	Fluid delivered (ml)	Time interval (min)	Per cent error
1	180	180	0.0
2	220	222	0.9
3	245	240	2.1
4	180	180	0.0
5	195	190	2.6
6	305	285	6.6
7	370	325	12.2
		Mean	3.5

Table 1 indicates the accuracy with which the apparatus will infuse solutions intravenously to individual animals over 45–325 minutes operating periods. Venous pressure is sufficiently constant to allow an even flow from the time of the initial adjustment.

Since the apparatus is all glass, with the exception of the indicated organic material (Fig. 1), which can be readily converted to glass, corrosion does not become a problem. Tolerances are wide enough to insure ready assembly from ordinary parts, but some attention must be given to the diameter and wettability of tube *B* to insure constant filling of the funnel.

If attention is given to stable placement of the cannula and elimination of foreign particles from the solutions, no difficulties are ordinarily encountered. Grit and large particulate matter, if present, will prevent the solenoid valve from seating properly and bring about an early empty-

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ing of the reservoir. Surgical gauze or other screening material can be introduced, if desired, into the funnel.

The total cost of special components for one animal is approximately forty dollars. Six or more additional animals can be simultaneously infused by the same timer at an approximate cost of fourteen dollars for each additional burette. Economy can be effected and the expense of the timer eliminated by use of a surplus 1-rpm motor mounted to actuate a microswitch by means of a heart cam or cam wheel.

SUMMARY

An apparatus for infusion at constant rate which can be assembled from inexpensive commercial components has been described.

REFERENCES

HUMPHLETT, W. J. (1955). An automatic constant-rate addition funnel. *Org. Chem. Bull.* **27** (3), 6.

MCKENNIS, H., JR., TURNBULL, L. B., and BOWMAN, E. R. (1957). γ-(3-Pyridyl)-γ-methylaminobutyric acid as a urinary metabolite of nicotine. *J. Am. Chem. Soc.* **79**, 6342-6343.

1003541609

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Grantee Publication #128

24. SYNTHESIS AND PROPERTIES OF PYRIDYLALANINES.

Herbert McKennis, Jr. and Edward R. Bowman; *Medical College of Virginia.*

To provide pyridylalanines for biochemical studies, methods for the synthesis of the parent compound and derivatives have been explored. 3-Pyridinecarboxaldehyde was condensed with creatinine in the presence of acetic anhydride and sodium acetate in glacial acetic acid The resulting 4-3'-pyridylmethylene-5-acetylcreatinine, m.p. 235-237°, on reduction with zinc dust and acetic acid gave 4-3'-pyridylmethylcreatinine, m.p. 275-279° in 80% yield. Upon treatment with strong barium hydroxide and heat this compound readily yielded N--methyl-β-3-pyridylalanine. The corresponding pyridylmethylhydantoin similarly and in contrast to published reports [Niemann, et. al., J. Am. Chem. Soc. 64 1678 (1942)] was hydrolysed with no unusual difficulty under the same conditions.

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TRC Grant #128

Reprinted from FEDERATION PROCEEDINGS
Volume 18 No. 1 *March 1959*

Made in United States of America

1462

OXIDATION OF COTININE IN VIVO. Edward R. Bowman* Lennox B. Turnbull* and Herbert McKennis, Jr. Department of Pharmacology, Medical College of Virginia, Richmond.

The metabolism of nicotine in the human and the anesthetized dog leads to the formation of desmethylcotinine, hydroxycotinine and other metabolites as shown in previous studies from this laboratory. Purification of the cotinine (5-(3'-pyridyl)-1-methylpyrrolidone-2) fraction of dog urine on Dowex 1(OH^-) has led to evidence for the presence of three previously undescribed Koenig positive components, with Rf 0.05, 0.11 and 0.17 (benzene-methanol-acetate pH 5.6) (60:15:25). Following administration of cotinine the material with Rf 0.05 has been obtained in crystalline form. It is optically inactive, reacts with carbonyl reagents and gives a strong Koenig reaction. These properties together with amphoteric behavior towards ion exchange resins point to the probable occurrence of ketocotinine as a metabolite. Oxidation of cotinine, therefore, proceeds by a variety of routes which provide pathways for the metabolic disposal of nicotine in the organism.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TRC Grant #128

[Reprinted from the Journal of the American Chemical Society, 80, 6597 (1958).]

Metabolism of Nicotine to (+)-gamma-(3-Pyridyl)-gamma-methylaminobutyric Acid

By Herbert McKennis, Jr., Lennox B. Turnbull and Edward R. Bowman

1003541612

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

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[Contribution from the Department of Pharmacology, Medical College of Virginia]

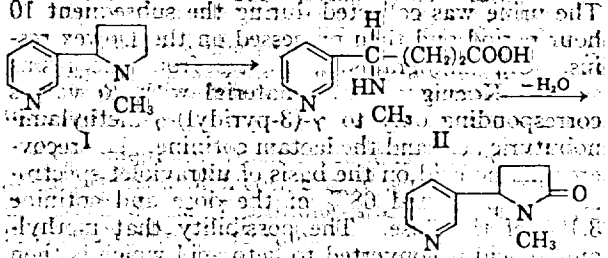
Metabolism of Nicotine to (+)-γ-(3-Pyridyl)-γ-methylaminobutyric Acid[1]

By Herbert McKennis, Jr.,[2a] Lennox B. Turnbull and Edward R. Bowman[2b]

Received April 11, 1958

Metabolism of (−)-nicotine in the dog leads to the formation of (+)-γ-3-(pyridyl)-γ-methylaminobutyric acid and cotinine. Under conditions of physiological *p*H, γ-3-(pyridyl)-γ-methylaminobutyric acid spontaneously cyclizes to the lactam cotinine. The reverse reaction, hydrolysis of the cotinine, does not occur appreciably under comparable conditions. The evidence suggests that an enzyme system is not obligatory for formation of cotinine *per se* and that a hydrolytic enzyme is required if cotinine is a natural precursor of γ-(3-pyridyl)-γ-methylaminobutyric acid.

In a previous communication from this Laboratory[3] data were presented to show that γ-(3-pyridyl)-γ-methylaminobutyric acid arises during the metabolism of nicotine in the dog. The appearance of this acid authenticated in part a general hypothetical route for the metabolism of nicotine which has been discussed previously.[4] It is now shown that both γ-(3-pyridyl)-γ-methylaminobutyric acid and its lactam cotinine arise from nicotine under physiological conditions. The relationships between nicotine and its metabolites which are experimentally verified as a result of these findings are represented below



I II III

Following intravenous administration of (−)-nicotine (I) the urine of dogs was acidified to *p*H 2 and then placed on a Dowex 50 (H^+) column which retained all of the Koenig positive material in the urine. Elution with *N*/1 ammonia water removed the Koenig positive material (disclosed with *p*-aminobenzoic acid and cyanogen bromide) which could be separated into six zones upon paper chromatography with ammonia–ethanol–butanol. These zones, which were absent from control urine, were compared for position by cochromatography with nicotine and a number of its derivatives. The results indicated the presence of nicotine (R_f 0.86), which had previously been reported to be present in urine by many authors.[5,6] The comparison suggested also that the material at R_f 0.73 was cotinine (III) and that the material at R_f 0.15 was γ-3-pyridyl-γ-methylaminobutyric acid (II).

Chloroform extraction of the ammoniacal solution of Koenig positive compounds removed the three faster moving components (nicotine, cotinine and unknown at R_f 0.61). The aqueous residue was then placed upon Dowex 1 (OH^-). The component with R_f 0.15 was retained. Components with R_f 0.38 and R_f 0.46 were not retained on the resin and are, hence, pyridine compounds with no or only feebly acidic functions. In addition it was determined through extraction of the zones from paper chromatograms that all of the substances in ethanolic HCl had single absorption maxima at 260 mμ. This places the metabolites as a group in a class of pyridine compounds with no side chain double bonds conjugated with the pyridine ring.[7] If one assumes a common molecular extinction coefficient, it can be seen (Table I) that the five metabolites account in one animal for approximately 21% of the administered dose of nicotine (153 mg.). The greatest quantity of material (9.2%) appeared at R_f 0.15. This R_f value corresponded to that of authentic γ-(3-pyridyl)-γ-methylaminobutyric acid. Further the material on heating became chloroform soluble and changed in R_f value to that of cotinine. These findings led to an unequivocal chemical identification.

Table I

Metabolism of Nicotine (153 Mg. I. V.) by Male Dog (15.3 Kg.)

	R_f[a] value of metabolite in Dowex 50 eluate	Mole,[b] % of dose
(1)	0.15	9.21
(2)	.38	5.40
(3)	.46	0.88
(4)	.61	2.79
(5)	.73	2.84

[a] Values were determined in the ammonia–ethanol–butanol system on Whatman No. 1 paper by the descending method at room temperature. For spectroscopic investigation areas corresponding to the Koenig positive zones were localized as quenching spots in ultraviolet light. R_f value of zone 1 corresponds to γ-(3-pyridyl)-γ-methyl-aminobutyric acid and R_f value of zone 5 corresponds to cotinine simultaneously chromatographed. The influence of temperature, paper and other factors on R_f values and the homogeneity of the zones have not been systematically investigated. R_f values were generally consistent to ± 0.05 unit but occasional aberrant values have been encountered. Control urine yielded no positive zones. [b] Calculated from optical density of eluted chromatographic zones at 260 mμ (in 0.1 *M* HCl in 95% ethanol)—optical density of eluate from metabolite free paper extracted in a similar fashion, using ε 4820.[5b]

(1) Presented in part at the Symposium on Tobacco Chemistry, Southeastern Regional Meeting of the American Chemical Society, Durham, North Carolina, November 15, 1957.

(2) (a) Appreciation is expressed to the Tobacco Industry Research Committee and the American Tobacco Company for support of this investigation. (b) Public Health Research Fellow of the National Heart Institute.

(3) H. McKennis, Jr., L. B. Turnbull and E. R. Bowman, This Journal, 79, 6342 (1957); Abstracts, Southeastern Regional Meeting, American Chemical Society, November 14–16, 1957, p. 23.

(4) H. McKennis, Jr., L. B. Turnbull, H. N. Wingfield, Jr., and L. J. Dewey, This Journal, 80, 1634 (1958).

(5) A. C. Corcoran, O. M. Helmer and I. H. Page, *J. Biol. Chem.*, 129, 89 (1939); J. K. Finnegan, P. S. Larson and H. B. Haag, *J. Pharmacol. Exp. Therap.*, 91, 357 (1947).

(6) P. S. Larson and H. B. Haag, *ibid.*, 76, 240 (1942).

(7) M. L. Swain, A. Bisner, C. F. Woodward and B. A. Brice, This Journal, 71, 1841 (1949).

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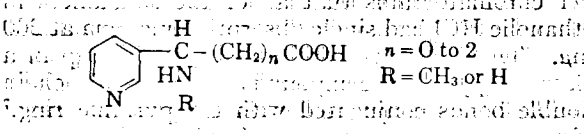
A Dowex 1 (OH^-) column which contained the R_f 0.15 material from the urine of six dogs was eluted with $N/1$ acetic acid. The eluate was concentrated to a brown sirup and heated to 155° under nitrogen to yield 45 mg. of oil which was soluble in chloroform. The oil yielded a crystalline picrate, m.p. 104–106°, which did not depress the melting point of authentic cotinine picrate and gave the correct analysis for C, H and N. The infrared absorption spectra of isolated and authentic picrates were identical. This established the metabolite as γ-(3-pyridyl)-γ-methylaminobutyric acid (II).

In previous studies[6,8,9] it was observed that following administration of nicotine, the urine of dogs contained material which gave a red color with cyanogen bromide without the addition of aromatic amine. Since this material could not be extracted from alkaline solution with ether and a variety of 3-pyridylalkanes were found to produce such a color if the carbon atom adjacent to the pyridine ring bore a primary or secondary amine, it was concluded that the data suggested the presence of an acid of the general formula



It was noted also that if the urine was boiled prior to reaction with cyanogen bromide the red color did not develop. *A priori*, if a single compound is involved in the reaction with cyanogen bromide, this compound is γ-(3-pyridyl)-γ-methylaminobutyric acid (n = 2, R = CH_3). The acid itself gives a direct red color with cyanogen bromide[16] and spontaneously or upon heating forms the lactam cotinine which does not give the color reaction. γ-(3-Pyridyl)-γ-aminobutyric acid (n = 2, R = H) gives a similar color reaction and forms the lactam desmethylcotinine[4] on heating. It is presumed from the current data that if γ-3-pyridyl-γ-aminobutyric acid is present in the urine it escaped detection in the procedures of isolation and chromatography employed in the present investigation. The lower homologs of the amino acids, where n = 0 to 1 and R = H or CH_3, although they may be present in the urine in small amounts, do not have properties which would readily explain the observed reactions. Where n = 0 no lactam can be formed and β-lactams, where n = 1, can be formed only with difficulty and under special conditions.[10]

Cotinine from the thermal cyclization of the metabolically formed γ-(3-pyridyl)-γ-methylaminobutyric acid displayed the same optical rotation as cotinine obtained from the oxidation of nicotine by Pinner's procedure[11] or cotinine resulting from the cyclization of γ-(3-pyridyl)-γ-methylaminobutyric acid which had been prepared by the published procedure.[4] Metabolic γ-(3-pyridyl)-γ-methylaminobutyric acid, therefore, has an approximate rotation of $[\alpha]D$ +17° and an asymmetric carbon atom with the same absolute configuration as that of (−)nicotine.[12]

Since it has been proposed[13] that γ-(3-pyridyl)-γ-methylaminobutyric acid can serve as an intermediate in the degradation of nicotine to γ-(3-pyridyl)-γ-oxobutyric acid, a bacterial metabolite of nicotine,[14,15] it was of interest to seek evidence for the keto acid in urine of nicotine-treated dogs. An examination of the paper chromatograms revealed no Koenig positive spot in the region of 0.24. If the keto acid had been present in the urine to the extent of 0.5% of the nicotine administered, the acid could have been easily detected by this procedure. The apparent lack of appreciable conversion of γ-(3-pyridyl)-γ-methylaminobutyric acid to γ-(3-pyridyl)-γ-oxobutyric acid is reaffirmed by a number of other observations. Following slow intravenous administration of 110 mg. of (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid to a dog[16] the urinary output of the compound, based on the cyanogen bromide color reaction, amounted to 86%. In our studies a dog was given (+)-γ-(3-pyridyl)-γ-methylaminobutyric acid in a single intraperitoneal dose. The urine was collected during the subsequent 10 hour period and then processed on the Dowex resins. Chromatograms of eluates from the resins showed Koenig positive material with R_f values corresponding only to γ-(3-pyridyl)-γ-methylaminobutyric acid and the lactam cotinine. The recovered amino acid on the basis of ultraviolet spectroscopy represented 68% of the dose and cotinine 3.1% of the dose. The possibility that methylamino acid is converted to keto acid which is then metabolized to the methylamino acid or other substances is, of course, not eliminated by the present studies. Experiments on the metabolism of γ-(3-pyridyl)-γ-oxobutyric acid are now in progress.

γ-(3-Pyridyl)-γ-methylaminobutyric acid, in the metabolism of nicotine, is on formal grounds both a precursor and metabolite of cotinine. A limited number of experiments were conducted to establish some of the factors concerned with this relationship. It was observed frequently during the course of the experiments that older samples of urine contained increasingly larger amounts of material in the cotinine zone of chromatogams and increasingly smaller concentrations of γ-(pyridyl)-γ-methylaminobutyric acid. This spontaneous lactamization is in agreement with the previously noted instability of the compound.[4] Since the urines of dogs in most of the experiments were excreted in the region of pH 5.2,[17] consideration was given to

(8) P. S. Larson, H. B. Haag and J. K. Finnegan, *J. Pharmacol. Exp. Therap.*, **86**, 239 (1946).

(9) P. S. Larson, *Ind. Eng. Chem.*, **44**, 279 (1952).

(10) S. A. Ballard, D. D. Melstrom and C. W. Smith in "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson and R. Robinson, Editors, Princeton University Press, Princeton, N. J., 1949, p. 973; J. C. Sheehan and E. J. Corey, *Organic Reactions*, **9**, 388 (1957).

(11) A. Pinner, *Ber.*, **26**, 292 (1893).

(12) It is assumed that in the preparation of cotinine from nicotine by the bromination procedure[11] there has been no inversion in the 2-position. There have been no experimental determinations of the absolute configuration of cotinine and only one of nicotine [P. Karrer and R. Widmer, *Helv. Chim. Acta*, **8**, 364 (1925)].

(13) E. Werle, H. Schievelbein and D. Spieth, *Arzneimittel-Forsch.*, **6**, 322 (1956).

(14) E. Wada and K. Yamasaki, THIS JOURNAL, **76**, 155 (1954).

(15) T. Tabuchi, *J. Agr. Chem. Soc. Japan*, **29**, 222 (1955).

(16) F. B. Owen, Jr., and P. S. Larson, *Arch. int. Pharmacodyn.*, **115**, 402 (1958).

(17) The urinary pH influences the amount of lactamization and cotinine formation *in vivo* and *in vitro* and bears also upon the amount of materials which may be reabsorbed from the urinary tract for subsequent additional exposure to enzymatic processes. Travell (*Proc.*

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the possibility that cotinine was entirely an artifact which arose during the collecting and processing of the urine. Studies (Table II) on the effect of *p*H on the stability of γ-(3-pyridyl)-γ-methylaminobutyric acid showed that within 0.5 hr. at 20° and *p*H 7 significant amounts of cotinine were formed. Even at *p*H 8.9 the amino acid in solution when heated to 100° is readily converted to cotinine. In contrast no cotinine was converted to amino acid in the *p*H range studied. These results indicate that, irrespective of initial mode of formation, some γ-(3-pyridyl)-γ-methylaminobutyric acid will serve as a precursor of cotinine in the biological systems studied. It is further concluded that any major direct conversion of cotinine to γ-(3-pyridyl)-γ-methylaminobutyric acid would depend upon the presence of an enzymatic system.

TABLE II

STABILITY OF AQUEOUS SOLUTIONS (2.86×10^{-5} *M*) OF γ-(3-PYRIDYL)-γ-METHYLAMINOBUTYRIC ACID

*p*H 0.1 *M* Na phosphate	Products after 1 hr.[a,b] 22°	100°
4.25	Acid > cotinine	acid = cotinine
6.40	Acid > cotinine	acid > cotinine
7.00	Trace cotinine	acid > cotinine
8.92	No cotinine	acid > cotinine

[a] The amount of product was estimated by paper chromatography and the Koenig reaction. [b] Cotinine ($3–4 \times 10^{-5}$ *M*) yielded no detectable amount of amino acid under these conditions.

Experiments in which cotinine is supplied to the animal instead of nicotine may serve to elucidate the possible role of cotinine as an intermediate in the metabolism of nicotine.

Acknowledgment.—The authors wish to thank Mr. Quentin S. McKennis and Mr. James Harlan for valuable assistance.

Experimental

Intravenous Administration of Nicotine to Mongrel Dogs.—Throughout an 8-hour period a solution of nicotine in 0.9% sodium chloride was infused into the femoral vein at a rate of 1 ml./min. The nicotine content of the saline was adjusted so that each animal under anesthesia received a total dose of 10 mg./kg. Urine was collected through a catheter during the infusion and during the subsequent 10-hour period.

Processing of Dog Urine for Nicotine Metabolites.—The pooled urine (1438 ml. representing 670 mg. of nicotine) was filtered through diatomaceous earth (Celite), and the *p*H was adjusted to *p*H 2 with 5 *N* HCl. The acidified urine was then placed on a Dowex 50 × −4 (H+) column (14 × 2.5 cm.). The column was washed with water (approx. 2 l.) until washings were virtually colorless. The column was then eluted with *N*/1 ammonia water (approx. 150 ml.) until no more Koenig positive material was obtained. The yellow brown solution was evaporated to dryness at room temperature. The residue was triturated with absolute ethanol (30 ml.). The alcoholic solution was filtered.[20] The filtrate was treated with decolorizing carbon and then concentrated to dryness. The residue was dissolved in 30 ml. of distilled water. The aqueous solution was extracted continuously with chloroform for 20 hr. After removal of chloroform under diminished pressure the extract weighed 118 mg. After a further treatment with decolorizing carbon the aqueous phase was adjusted to *p*H 10–11 with ammonia water and then placed on a Dowex 1 (OH) column. The effluent contained Koenig positive material which was combined with Koenig positive material obtained by washing the column with water. This fraction weighed 1025 mg. after removal of the solvent. The column was then eluted with *N*/1 acetic acid until no more Koenig positive material could be obtained. This solution upon concentration to dryness at room temperature yielded 383 mg. of brown solid (Fraction C) with Koenig positive material at R_f 0.15, corresponding to γ-(3-pyridyl)-γ-methylaminobutyric acid. All chromatograms were conducted by the descending method on Whatman No. 1 paper at room temperature with ammoniacal alcohol (1 vol. *N*/2 ammonia water, 1 vol. 95% ethanol, 4 vol. 1-butanol).

Identification of γ-(3-Pyridyl)-γ-methylaminobutyric Acid in Fraction C.—The above obtained 383 mg. of brown solid was heated to 155° in an atmosphere of nitrogen for 15 minutes and then dissolved in 15 ml. of water. After a treatment with decolorizing carbon, the clear solution was extracted with six portions (15 ml.) of chloroform. The residue, upon removal of the chloroform, weighed 43 mg. (λ_{max} 262 mμ in ethanol). In paper chromatograms the oil yielded a single Koenig positive zone corresponding to cotinine (R_f 0.73).

Cotinine Picrate.—A sample of (−)cotinine was treated with an excess of a saturated methanolic solution of picric acid. After several days in the refrigerator the crystalline residue was collected and recrystallized from 95% ethanol (m.p. 104–106°). For analysis the sample was dried at 1 mm. over KOH.

Anal. Calcd. for $C_{18}H_{15}N_9O_8$: C, 47.41; H, 3.73; N, 17.28. Found: C, 47.45; H, 3.80; N, 17.34.

Cotinine Picrate from Metabolic γ-(3-Pyridyl)-γ-methylaminobutyric Acid.—The 43-mg. sample of cotinine from the urinary methylamino acid was treated with an excess of methanolic picric acid. The precipitate after four recrystallizations from 95% ethanol weighed 21 mg. (m.p. 104–106°). Admixture with authentic cotinine picrate caused no depression of the melting point. In Nujol mulls the infrared absorption spectra of authentic and isolated samples were identical.[21]

Anal. Calcd. for $C_{18}H_{15}N_9O_8$: C, 47.41; H, 3.73; N, 17.28. Found: C, 47.49; H, 3.80; N, 17.19.

Formation of γ-(3-Pyridyl)-γ-methylaminobutyric Acid by Male Dog.—Since previous studies on nicotine metabolism were accomplished largely on female dogs, urine from a male dog (12.8 kg.) which had received nicotine as described above, was processed on Dowex 50 as described above. The solids from the chloroform extract, which contained cotinine, nicotine and unidentified metabolite(s), weighed 128 mg. Cyclization of the Dowex 1 eluate which contained γ-(3-pyridyl)-γ-methylaminobutyric acid resulted in 9.4 mg. of cotinine with extinction coefficient at 260 mμ in 0.1 *M* HCl in 95% ethanol corresponding to a calculated 9.02 mg. of cotinine. On a molar basis this represents 6.9% of the dose of nicotine. A similar experiment conducted with a female dog (14.1 kg.) resulted in the isolation of 3.6 mg. of thermally formed cotinine, identified by paper chromatography and ultraviolet spectrum, corresponding to 3.8 mg. of γ-(3-pyridyl)-γ-methylaminobutyric acid, or 2.32% of the dose of nicotine.

Metabolism of γ-(3-Pyridyl)-γ-methylaminobutyric Acid in the Dog.—An unanesthetized male mongrel dog (9.0 kg.) was given 810 mg. of γ-(3-pyridyl)-γ-methylaminobutyric acid intraperitoneally. The urine was collected in the ensuing 22-hour period. The Koenig positive fraction from the Dowex 50 column was adjusted to a volume of 50 ml. An aliquot (0.1 ml.) was chromatographed on paper. Only two Koenig positive spots (corresponding to γ-(3-pyridyl)-γ-methylaminobutyric acid and cotinine) were obtained. The Koenig positive areas were eluted with 0.1 *M* HCl in 95% ethanol. The ultraviolet absorption spectra of these

Soc. Exper. Biol. Med., **45**, 552 (1940)) noted that alkalinity favored absorption of nicotine from the bladder of the cat. Others[18,19] have considered the effect of *p*H on reabsorption in humans.

(18) J. Travell, O. Bodansky and H. Gold, *J. Pharmacol. Exp. Therap.*, **69**, 307 (1940).

(19) H. B. Haag and P. S. Larson, *ibid.*, **76**, 235 (1942).

(20) In some experiments it was found that additional Koenig positive material can be recovered from the residue by water extraction.

(21) We thank Mr. W. B. Wartman, Jr., of the Research Laboratory, American Tobacco Company, for these determinations. When the spectra were determined in KBr pellets, small differences were noted in the region above 7 μ. Since both isolated and authentic specimens behaved similarly in this regard the differences are attributed to polymorphism or possible crystal orientation effects.

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indicated an excretion of 68% of the amino acid unchanged and 3.1% of the amino acid appeared as cotinine.

Thermal Lactamization of Synthetic and Metabolic γ-(3-Pyridyl)-γ-methylaminobutyric Acid.—The monohydrate of the methylamino acid[4] (100 mg.) ($[\alpha]^{22}D$ +17.2) in a Pyrex tube was heated for 0.5 hr. in an atmosphere of nitrogen at 145–150°. The oily residue was extracted with six portions of chloroform (5 ml. each). The product was dissolved in methanol and gave an optical rotation ($[\alpha]^{21.5}_{5461}$ −18.16° (*c* 4.35)).

Fraction C (303 mg.) from the pooled urine of six dogs (893 mg. of administered nicotine) was heated for 0.5 hr. at 145–150° under nitrogen. The residue then was dissolved in 5 ml. of distilled water. The solution was extracted with six portions (5 ml. each) of chloroform. Upon evaporation of the chloroform extract under diminished pressure a residue of cotinine (49.8 mg.) or 5.55% of the dose of nicotine was obtained ($[\alpha]^{30}_{5461}$ −18.77° (*c* 4.98)). In comparison a sample of cotinine prepared from nicotine by the method of Pinner[11] had a specific rotation ($[\alpha]^{30}_{5461}$ −19.85; (*c* 5.59, methanol)).

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METABOLISM OF NICOTINE IN THE HUMAN AND EXCRETION OF PYRIDINE COMPOUNDS BY SMOKERS

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METABOLISM OF NICOTINE IN THE HUMAN AND EXCRETION OF PYRIDINE COMPOUNDS BY SMOKERS[1]

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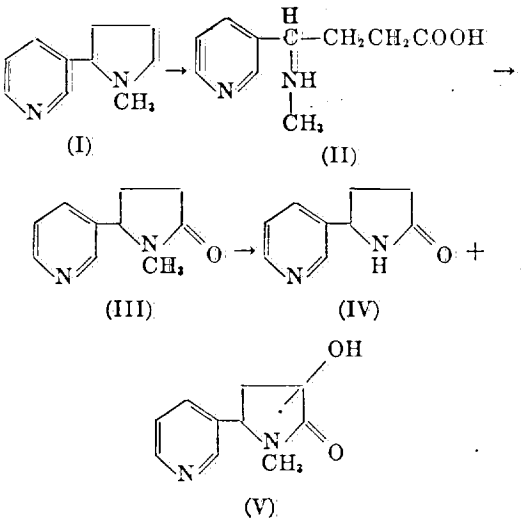
On many occasions, such as in industrial accidents or inadvertent overexposure to insecticides, in diagnostic procedures for diabetes insipidus (Chalmers and Lewis, 1951; Cates and Garrod, 1951; Dingman *et al.*, 1957) or in the enjoyment of tobacco smoking, the human assimilates nicotine in varying quantities. It has been frequently observed that following the smoking of tobacco only a small fraction of the absorbed nicotine is eliminated in the urine (Corcoran *et al.*, 1939). It may be deduced, on the basis of animal experiments, that this low excretion of nicotine probably arises from an active metabolism and does not indicate storage or excretion by other pathways.

Studies in the rat (Ganz *et al.*, 1951) and in the dog (Bennett *et al.*, 1954) with uniformly labeled C^{14} nicotine have demonstrated that the major part of the administered radioactivity is eliminated in the urine. In the dog the unchanged nicotine in the urine accounts for approximately 10% of the administered dose.

Examination of the urine of dogs after intravenous administration of *l*-nicotine (I) has led (McKennis *et al.*, 1957–1959) to the isolation and identification of a variety of metabolites including γ-(3-pyridyl)-γ-methylaminobutyric acid (II) and its lactam cotinine (III), desmethylcotinine (IV) and hydroxycotinine (V). γ-(3-Pyridyl)-γ-methylaminobutyric acid can under conditions of physiological pH and temperature (McKennis *et al.*, 1957) spontaneously lactamize to yield cotinine, and the latter gives rise to both desmethylcotinine and hydroxycotinine when administered to the dog (McKennis *et al.*, 1958b, 1959). The known metabolic interrelationships are summarized.



(I) (II) (III) (IV) (V)

The development of the procedures used in the foregoing have made possible a study of nicotine metabolism in the human. This communication describes studies on the isolation of some of the chloroform-soluble metabolites of nicotine present in the urine of the human (non-smoker) and of pyridine compounds present in the urine of human smokers.

METHODS AND MATERIALS. *Administration of nicotine to the human.* A normal adult male (80.4 kg) (non-smoker) received an oral dose of 30 mg of *l*-nicotine per day (in consecutive hourly daytime doses of 3 mg each in gelatine capsules). The experimental period was concluded at the end of the third day because of nausea and the accompaniment of vomiting on the commencement of the fourth day.

Procedure for the isolation of the metabolites. The combined total urine volume (5 liters) from the 3-day period was adjusted to pH 10 with ammonia water and then exhaustively extracted with chloroform. The residue after the evapora-

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[2] Public Health Research Fellow of the National Heart Institute, National Institutes of Health, U. S. Public Health Service.

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tion of the chloroform was chromatographed on Whatman no. 1 paper with 0.5 N ammonia water (1 volume)-95% ethanol (1 volume)-n-butanol (4 volumes) (McKennis *et al.*, 1957). The Koenig positive zones were located with a spray of alcoholic *p*-aminobenzoic acid which was followed by treatment with vapors of cyanogen bromide. Paper chromatograms were also prepared with *sec.* butanol-formic acid-water (Hausman, 1952).

The residue from the chloroform extract was dissolved in 25 ml of water and adjusted to pH 2 with 5 N HCl. The solution was passed through Dowex 50 (H^+) (20 × 150 mm). After a water wash the column was eluted with 80 ml of N/1 ammonia water. The ammoniacal solution was concentrated to a brown residue. A solution of the latter in 10 ml of water was adjusted to pH 9 with N/1 ammonia water and passed through Dowex 1 (OH^-) (15 × 100 mm). The effluent and water wash were concentrated to a brown gum (149 mg). Elution of the column with N/1 acetic acid served to remove a component with an R_f value of 0.74 (ammonia-ethanol-butanol). An extract of the gum in 23 ml of chloroform was poured onto an acid-washed alumina column (5 g) and eluted with ether containing successively increasing concentrations of methanol. The fractions, obtained with 1 to 10% methanolic ether, containing a single Koenig component at R_f 0.74 (ammonia-ethanol-butanol), were combined. Upon evaporation of the solvent 10.1 mg of cotinine were obtained as a light colored oil (representing about 10% of the administered nicotine dose), λ max 260 mμ in ethanolic HCl. The oil was converted to 21.5 mg of cotinine dipicrate, micro m.p., 102.5°C.

Fractions with R_f 0.61 (ammonia-ethanol-butanol), obtained from the alumina column (above) with 10 to 100% methanol, were combined and evaporated to give 14.7 mg of gum. The latter, after treatment with acetic anhydride-pyridine and upon evaporation of the reagents, was rechromatographed on alumina as described above. Two fractions R_f 0.61 (1.62 mg) and R_f 0.75 were obtained.

Treatment of smokers' urine. Smokers' urine (60 liters) was obtained by voluntary daytime contributions from male laboratory workers. Chloroform extraction of the urine was accomplished in the previously described manner. The residue from the evaporation of the chloroform was treated with water and methanol to obtain all of the Koenig positive material in solution. Cotinine (87 mg) was isolated and then converted to the dipicrate by the procedure described above.

Examination of human urine for 3-aminomethylpyridines. Samples of urine from smokers or the subject who ingested nicotine were adjusted to pH 2 and placed on Dowex 50 (H^+). The effluent and an ammoniacal eluate of the resin column were treated with cyanogen bromide (Larson and Haag, 1942) before and after boiling.

Preparation of cotinine dipicrate. A solution of synthetic cotinine (500 mg, 0.0029 mol) (McKennis *et al.*, 1959) in 1 ml of methanol, and 1.6 g (0.007 mol) of picric acid was heated to boiling. Upon cooling and scratching, a yellow crystalline precipitate formed. The product had a constant micro m.p. (102.5°C) after 6 recrystallizations from methanol. Calculated for $C_{22}H_{18}N_8O_{15}$: C, 41.65; H, 2.86; N, 17.66. Found: C, 42.37; H, 2.89; N, 17.97.

Cotinine monopicrate from cotinine dipicrate. Cotinine dipicrate was dissolved in a minimum amount of hot absolute alcohol. The solution upon cooling deposited yellow crystals. After 6 recrystallizations the micro m.p. was 104°–105°C. Calculated for $C_{16}H_{15}N_5O_8$: C, 47.41; H, 3.73; N, 17.28. Found: C, 47.30; H, 3.75; N, 17.16.

RESULTS. During the 3-day period the human subject excreted approximately 10% of the administered nicotine in the form of cotinine. Due to the fact that tobacco smokers' urine was obtained only during the daytime and without data on the smoking habits of the subjects or composition of the smoke it is not possible to calculate percentage of metabolism. Cotinine, in addition to other pyridine compounds, has been identified in tobacco smoke (Quin, 1958; Rayburn, personal communication, 1958).

The chromatographic evidence (table 1) points to the presence of cotinine, desmethylcotinine and hydroxycotinine in all samples examined. Positive identification of cotinine was effected by infrared spectrograms of the picric acid salts of cotinine. Double verification was made by converting dipicrates to monopicrates. The dipicrate was identical to a sample prepared in the laboratory of the Japan Monopoly Corporation (Wada *et al.*, 1959). Authentic salts and corresponding isolated salts had the same melting point. The mixed melting points showed no depression.

The cyanogen bromide reaction of Dowex 50 (H^+)-treated urine was negative. The ammoniacal eluate of the resin was positive and gave a pink color. Following heat treatment, which cyclizes γ-(3-pyridyl)-γ-methylaminobutyric acid to cotinine, the pink color was no longer obtained on test samples.

DISCUSSION. The results obtained in the survey of nicotine metabolism in the human indicate a

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TABLE I

*R_f values of the chloroform-soluble, Koenig positive components of the urine from the human (non-smoker), human smoker and the dog**

Source	R_f Values	
	Ammonia-ethanol-butanol	*sec*-Butanol-formic acid-water
Human (non-smoker)	0.61§ and 0.74‡	0.21§ and 0.37‡
Human smoker	0.61,§ 0.74,‡ 0.85,† 0.90	0.21§ and 0.37‡
Dog	0.61,§ 0.74,‡ 0.85†	0.21§ and 0.37‡

* Bladder urine collected from dogs (receiving 10 mg/kg nicotine by intravenous infusion over an 8-hr period) via indwelling catheters. Control urines of both species yielded no Koenig positive zones. Nicotine was administered orally to the human subject. Pooled urine from male smokers was employed.

† Cochromatographed with *l*-nicotine.

‡ Cochromatographed with cotinine.

§ Cochromatographed with desmethylcotinine. Fraction acetylated to give components corresponding to acetoxycotinine and desmethylcotinine.

similarity to the previous results obtained in the dog. Chemical isolation of cotinine as one of the metabolites in both species points to a desirability of a study of cotinine metabolism in the human. It has been established that cotinine serves as an intermediate in the degradation of nicotine in the dog. The paper chromatograms of the chloroform-soluble fraction of human urine strongly suggest that cotinine has a similar role in man. Since the desmethylcotinine fraction (R_f 0.61, ammonia-ethanol-butanol) of urine yields on acetylation material with chromatographic characteristics of desmethylcotinine and acetoxycotinine, it is inferred that the pathway of nicotine metabolism in the human may resemble that of dog, *viz.*, nicotine → cotinine → hydroxycotinine plus desmethylcotinine.

Similar pathways are inferred also from a study of the urine of smokers (table 1). Both dog urine and smokers' urine contain material which is Koenig positive at R_f 0.85 (ammonia-ethanol-butanol) corresponding to nicotine. Other investigators have noted the urinary excretion of nicotine under comparable conditions. The human subject who received nicotine excreted, in contrast, no nicotine detectable under the conditions employed for isolation. One explanation for this difference may lie in the differences in route of administration. In both the smoker and the dog receiving nicotine intravenously it is anticipated that some nicotine may be presented to the kidney for excretion without opportunity for the liver (Hucker *et al.*, 1959) or other tissues (Akira, 1957; Miller and Larson, 1953; Werle and Uschold, 1948) to degrade the compound. In contrast, absorption of orally administered nicotine may provide, via portal circulation, a unique opportunity for metabolic transformation in the liver.

The component in smokers' urine at R_f 0.90 (ammonia-ethanol-butanol), which was not encountered in either dog urine or human urine after nicotine, may represent a new nicotine metabolite. On the other hand, pyridine compounds other than nicotine in smoke may be responsible for appearance of the material. It is possible, too, that, unbeknown to us, some of the volunteers may have been receiving medication which is responsible for this component.

Although the present study does not directly concern itself with the immediate precursors of cotinine some conclusions may be drawn with respect to the source of this compound. γ-(3-Pyridyl)-γ-methylaminobutyric acid, which is responsible for a pink color produced by addition of cyanogen bromide to the urine of the nicotine-treated dog, is spontaneously lactamized to cotinine under physiological conditions of pH and temperature (McKennis *et al.*, 1958a). Werle *et al.* (1956) have reported that smokers' urine gives the color reaction with cyanogen bromide. In the present study material obtained, following smoking or ingestion of nicotine, by eluting pyridine components of the urine from Dowex 50 (H^+) showed a similar behavior with cyanogen bromide. The reaction could not be obtained if the material was heated prior to addition of cyanogen bromide. This behavior parallels that of γ-3-pyridyl-γ-methylaminobutyric acid which loses its color-producing ability as the result of lactam formation (acylation of the methylamino group). It may be inferred then that in the human γ-3-pyridyl-γ-methylaminobutyric acid is a nicotine metabolite which lactamizes to cotinine.

Various studies *in vitro* provide evidence for an oxidative metabolism of nicotine to cotinine without necessity for the intermediate methylamino acid. Nicotine in the presence of peroxide or merely on storage (Frankenburg and Vaitekunas, 1957) is oxidized to cotinine. This suggests the possibility of catalase-peroxide catalysis in the formation of cotinine *in vivo*. Thus, at least two pathways to the formation of the important intermediate cotinine, one direct and the other

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via γ-(3-pyridyl)-γ-methylaminobutyric acid, are potentially available for physiological disposition of nicotine.

SUMMARY

Following the smoking of tobacco or ingestion of nicotine the urine of human subjects contained cotinine which was identified as the picrate and dipicrate. Chromatograms of the urines suggest the presence of hydroxycotinine and desmethylcotinine and related Koenig positive components and lead to the conclusion that cotinine plays, in man as well as in the dog, an important role as intermediate in nicotine metabolism.

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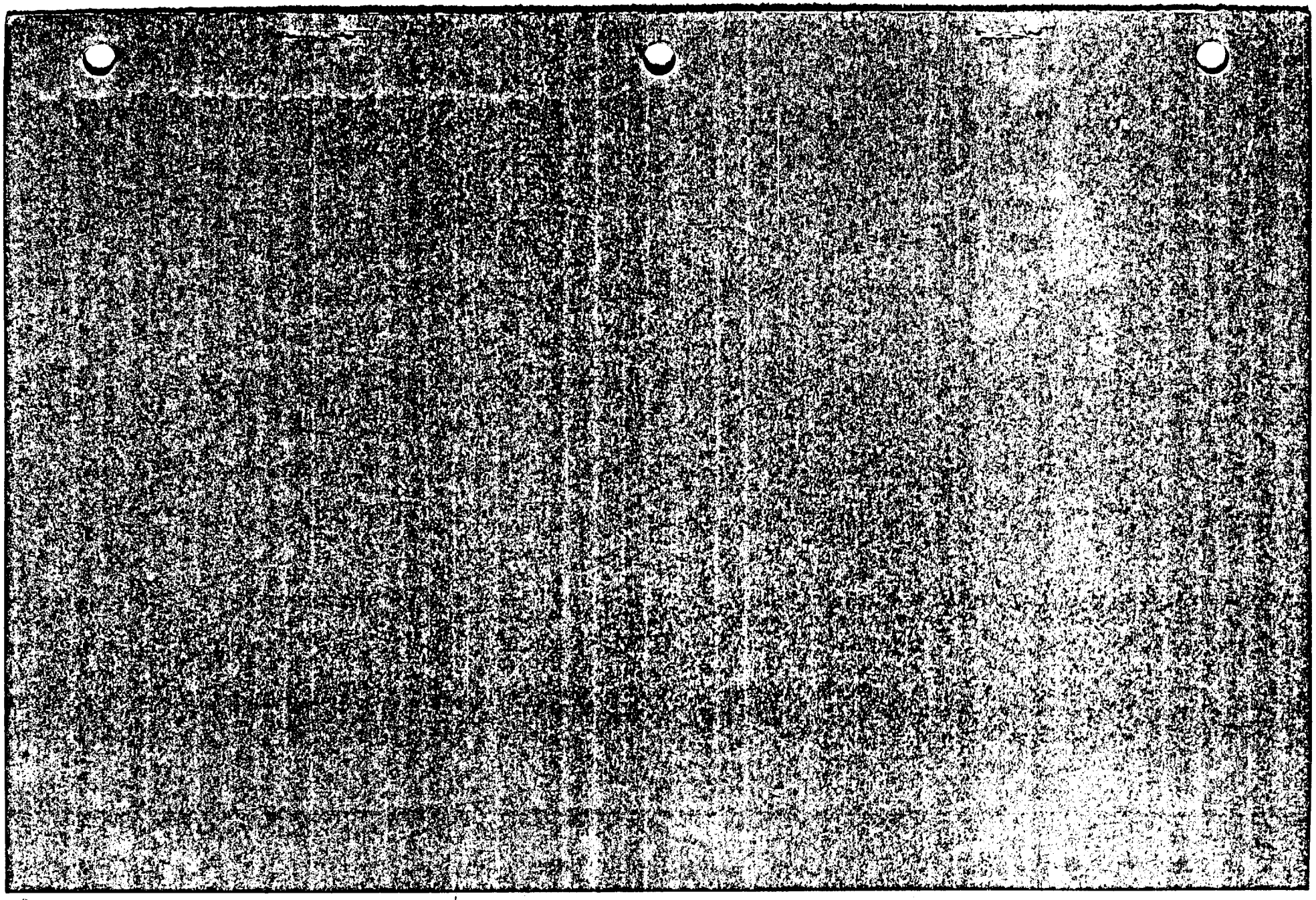

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Demethylation of Cotinine *in Vivo*

By Herbert McKennis, Jr., Lennox B. Turnbull, Edward R. Bowman and Einosuke Wada

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[Contribution from the Department of Pharmacology, Medical College of Virginia]

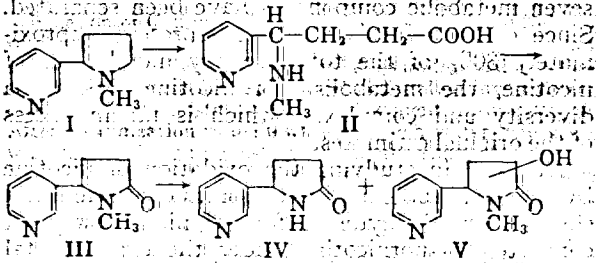
Demethylation of Cotinine *in Vivo*[1]

By Herbert McKennis, Jr.,[2] Lennox B. Turnbull, Edward R. Bowman[3] and Einosuke Wada[4]

Received February 5, 1959

Administration of (−)-cotinine, an intermediate in the metabolism of (−)-nicotine, leads to the excretion of (−)-desmethylcotinine, hydroxycotinine and other Koenig positive material in the urine of the dog. Reductive amination of γ-(3-pyridyl)-γ-oxobutyric acid in the presence of Raney nickel yielded racemic desmethylcotinine which was resolved to give (−)-desmethylcotinine corresponding to the metabolic product.

Following the administration of (−)-nicotine the cat,[5] the dog[6–7] and the human[8,9] excrete in the urine a variety of Koenig positive materials which are not found in control urine. One of the several Koenig positive zones from paper chromatography has been found to contain cotinine.[6–9] The latter has been identified[10] chemically with the isolation of cotinine picrate,[6–9] and another has been found to contain γ-(3-pyridyl)-γ-methylaminobutyric acid,[6,7] identified after thermal cyclization into the lactam cotinine.[6,7] This cyclization to cotinine takes place spontaneously at physiological *p*H. Cotinine, through its limited toxicity, is a convenient intermediate, therefore, for exploration of nicotine metabolism. The isolation of two new nicotine metabolites now permits representation of the cotinine pathway of nicotine metabolism in the abbreviated form



For the current studies attention was focused solely on the chloroform-soluble metabolites of nicotine (I) and cotinine in dog urine. (−)-Cotinine (III) was administered intravenously to male mongrel dogs under anesthesia. The urine was adjusted to *p*H 8–9 by addition of ammonia water and extracted continuously with chloroform. Samples from evaporation of the chloroform yielded Koenig positive zones at R_f 0.61 and R_f 0.74 upon paper chromatography with ammonia–ethanol–butanol.[11] The latter corresponded in R_f value to known cotinine upon cochromatography and the former corresponded in R_f value to material obtained from both dogs[6,7] and humans[8,9] following administration of (−)-nicotine (I).

The aqueous solution of the residue from evaporation of the chloroform was placed on Dowex 50 (H^+). The Koenig positive material was eluted with *N* ammonia water and further purified by passage through Dowex 1 (OH^-) which served to remove some Koenig negative solids as well as a component with R_f 0.75. The latter has an R_f value virtually indistinguishable from cotinine in many solvent systems but differs from it by an apparent amphoteric behavior displayed on ion-exchange resins. Investigations on this component are in progress.

The effluent from the Dowex 1 (OH^-) resin containing Koenig positive bases was reprocessed on Dowex 50 (H^+) for further purification. The solids from the latter were dissolved in chloroform and chromatographed on alumina and eluted with ether containing methanol in concentration increasing from 10–75% (v./v.). The 10% methanolic eluates contained material with R_f 0.74 corresponding to cotinine. The fractions removed with 11–75% methanol, although composed of both oil and crystals showed only a positive Koenig zone at R_f 0.61. Following treatment with acetic anhydride–pyridine the foregoing mixture was chromatographed on alumina and eluted with methanolic

(1) Presented in part at the IV International Congress of Biochemistry, Vienna, September 1–6, 1958.

(2) The authors are grateful to The American Tobacco Company and The Tobacco Industry Research Committee for generous support.

(3) Public Health Research Fellow of the National Heart Institute.

(4) American Tobacco Company Research Fellow.

(5) F. B. Owen, Jr., and P. S. Larson, *Arch. int. pharmacodyn.*, **115**, 402 (1958).

(6) H. McKennis, Jr., L. B. Turnbull and E. R. Bowman, This Journal, **79**, 6342 (1957).

(7) H. McKennis, Jr., L. B. Turnbull and E. R. Bowman, *ibid.*, **80**, 6597 (1958).

(8) E. R. Bowman, L. B. Turnbull and H. McKennis, Jr., *Virg. J. Sci.*, **9**, 438 (1958).

(9) E. R. Bowman, L. B. Turnbull and H. McKennis, Jr., *J. Pharmacol. Exp. Therap.*, in press.

(10) Paper chromatographic and ultraviolet absorption data have led to the suggestion (F. E. Guthrie, R. L. Ringer and T. G. Bowery, *J. Econ. Entom.*, **50**, 822 (1957), that cotinine arises from the metabolism of nicotine in insects.

(11) H. McKennis, Jr., L. B. Turnbull, H. N. Wingfield, Jr., and L. J. Dewey, This Journal, **80**, 1634 (1958).

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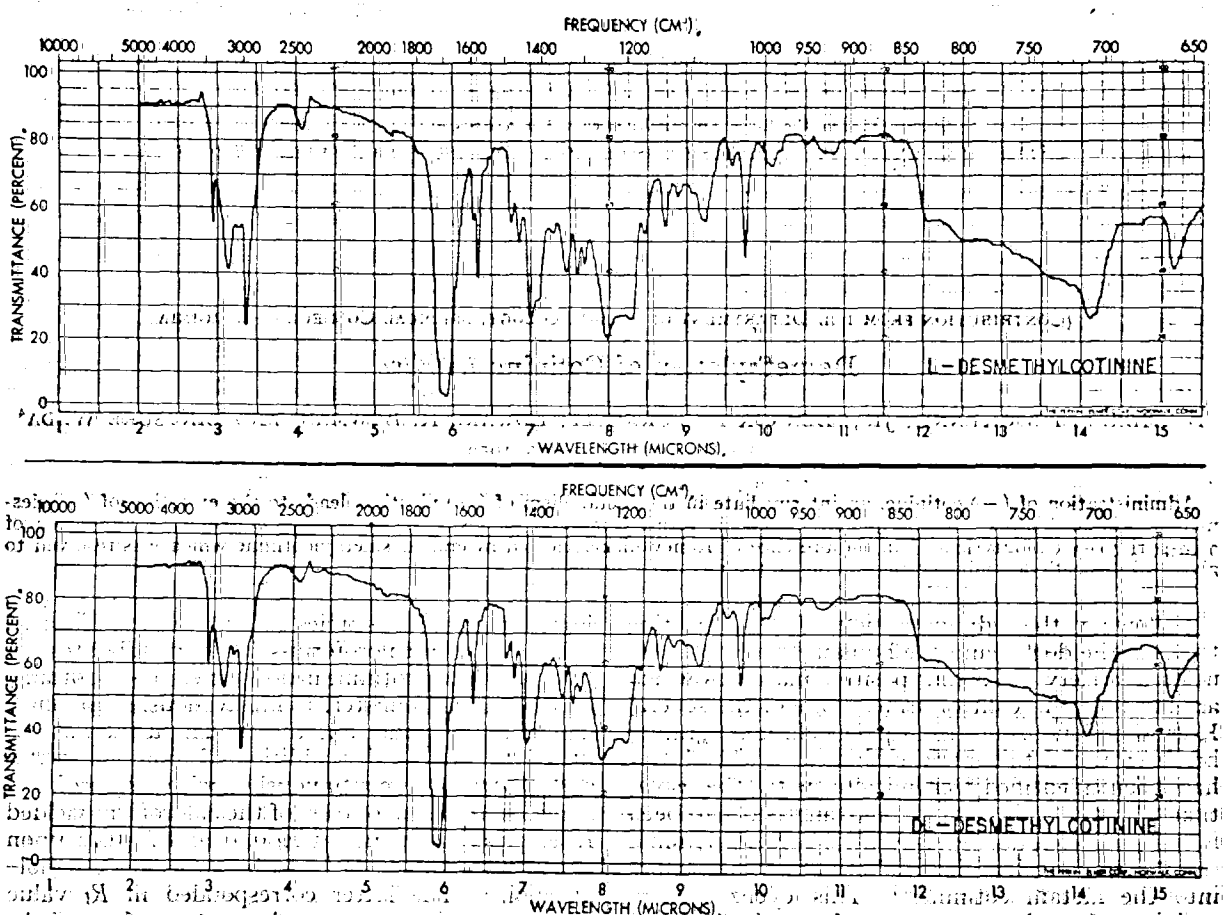

Fig. 1.—Infrared absorption spectra of natural and synthetic desmethylcotinine in chloroform solution.

ether to give two Koenig positive components (R_f 0.61 and R_f 0.75).

The material with R_f 0.61 readily crystallized from benzene to yield colorless crystals melting at 135–136°. The elementary analysis corresponded to that of desmethylcotinine (IV). The R_f value and infrared absorption spectra[12] in chloroform (Fig. 1) were in good agreement with synthetic *dl*-desmethylcotinine.

The acetyl derivative (R_f 0.75), which was obtained as an oil, yielded a yellow picrate, m.p. 166.5–170°. The elementary analysis agreed with the calculated value for the picrate of acetylated hydroxycotinine, $C_{18}H_{17}N_8O_{16}$. This has led us to adopt the trivial name hydroxycotinine for the metabolite (V). The strong Koenig positive reaction of the compound is substantial evidence to support assignment of the hydroxyl group to the pyrrolidone ring of cotinine. Exact assignment of position and structure of the molecule must await unequivocal synthetic and degradative studies.

The isolation of (−)-desmethylcotinine and hydroxycotinine as a result of the metabolism of cotinine experimentally affirms the importance of a role attributed to cotinine as an intermediate in nicotine metabolism.[11] It is also apparent that many new metabolic pathways for the degradation of cotinine must be considered. Owen and Larson[5] in their investigation of chromatograms of the urine of dogs after administration of C^{14}(−)-nicotine suggested the presence of three major and possibly four minor metabolites of nicotine. As a result of the present and previous studies,[6,7] seven metabolic components have been separated. Since on a molar basis these represent approximately 30% of the total urinary metabolites of nicotine, the metabolism of nicotine presents a diversity and complexity which is far in excess of the original estimates.

Hucker[13] in studying the oxidation of nicotine by fortified rabbit liver microsomes concluded that since no formaldehyde was formed, nicotine was not converted to nornicotine under the experimental conditions. If oxidative demethylation is involved in the metabolism of nicotine to desmethylcotinine, our isolation of desmethylcotinine points to the presence of a pathway for demethylation which may involve other cellular components of the liver[14,15] or other organs.[16] In the event, however, that demethylation occurs by transmethylation, rather than oxidative demethylation, formaldehyde cannot be anticipated as a primary metabolic product or serve as an absolute indicator of de-

(12) Kindly determined by Mr. J. Scott Osborne of The American Tobacco Company, Research Laboratory.

(13) H. B. Hucker, *J. Pharmacol. Exp. Therap.*, **122**, 33a (1958).

(14) T. Akira, *Folia Pharmacol. Japon.*, **53**, 553 (1957).

(15) E. Werle and R. Müller, *Biochem. Z.*, **308**, 355 (1941).

(16) See for example: A. W. Miller, Jr., and P. S. Larson, *J. Pharmacol. Exp. Therap.*, **109**, 218 (1953); E. Werle and E. Uschold, *Biochem. Z.*, **318**, 531 (1948).

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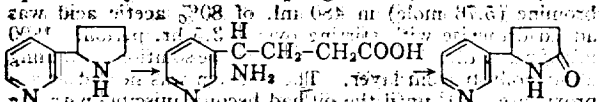
methylation.[17] Irrespective of the type of demethylation involved, the simplest explanation for the formation of desmethylcotinine involves only participation of cotinine or γ-(3-pyridyl)-γ-methylaminobutyric acid (II).

Werle, *et al.*,[18] as a result of studies on the metabolism of nicotine considered the possibility that nicotine was demethylated to give nornicotine (VI). Chemical verification of this proposal would suggest additional metabolic pathways to desmethylcotinine with nornicotine and possibly γ-(3-pyridyl)-γ-aminobutyric acid (VII) as intermediates and no involvement of cotinine. Experiments to test such a possibility are now in progress.



To facilitate further metabolic work improved procedures have been developed for the synthesis of cotinine and desmethylcotinine. The latter has been resolved successfully with *d*-10-camphorsulfonic acid. This resolution provides both optical antipodes. Reduction of the racemic mixture provided[11] synthetic routes to nornicotine. Racemic cotinine has been reduced to racemic nicotine.[19] (−)-Cotinine upon reduction with lithium aluminum hydride in the present study yielded (−)-nicotine. This reduction supplies experimental confirmation of the previous conclusion[7] that in the metabolism of nicotine to (+)-γ-3-pyridyl-γ-methylaminobutyric acid and (−)-cotinine the absolute optical configuration of (−)-nicotine is retained.

Acknowledgments.—The authors are grateful to James H. Smith, Covie E. See and Jeffrey S. McKennis for assistance.

Experimental[20]

Isolation of Cotinine Metabolites.—(−)-Cotinine (5.9 g.) was administered intravenously[21] (100 mg./kg.) during an 8-hour period to two male mongrel dogs under Dial anesthesia. Urine was collected through an indwelling catheter during the administration and subsequent 10-hr. period. After alkalinization to *p*H 9 with ammonia water, the urine was extracted continuously with chloroform. The residue from evaporation of the chloroform (3.1 g. of brown gum) yielded two Koenig positive zones (R_f 0.74 and R_f 0.61, ammonia–ethanol–butanol, R_f 0.48 and R_f 0.30, formic acid–*sec*-butyl alcohol–water). Upon cochromatography these zones corresponded to those previously obtained from dogs treated intravenously with nicotine or orally or intraperitoneally with cotinine. The brown gum was dissolved in 10 ml. of water and adjusted to *p*H 1.5. The solution was placed on Dowex 50 (H^+). After a water wash, Koenig positive material, which was retained on the resin, was eluted with *N* ammonia water. The residue (1.5 g.) from evaporation of this solution was dissolved in dilute ammonia and placed on a Dowex 1 (OH^-). The residue from concentration of the effluent was again placed on Dowex 50 (H^+). Elution with 0.5 *N* ammonia water served to remove 738 mg. of material which again gave Koenig positive zones (R_f 0.74 and R_f 0.61, ammonia–ethanol–butanol). A chloroform solution of the mixture was placed on acid-washed alumina and eluted with ether containing methanol increasing from 5–75% (v./v.). The 5–10% methanolic eluates contained material with R_f 0.74 corresponding to cotinine (299 mg.). The fraction removed with 11–75% methanol, although composed of both oil and crystals (359 mg.), showed only one Koenig positive zone (R_f 0.61, ammonia–ethanol–butanol; R_f 0.30, formic acid–*sec*-butyl alcohol–water).

Isolation of (−)-Desmethylcotinine and Acetylated Hydroxycotinine.—The mixture of oil and crystals was dissolved in pyridine containing an excess of acetic anhydride and allowed to stand overnight. The residue after removal of reagents at the vacuum pump showed the original R_f value plus a zone at R_f 0.75 (ammonia–ethanol–butanol). Rechromatography on acid-washed alumina served to separate the two fractions. The R_f 0.75 material appeared as a colorless oil from concentration of the eluates in 10% methanolic ether (75 mg.). The oil was treated with an excess of picric acid in methanol to yield a crystalline picrate which was purified for analysis by recrystallization from 95% ethanol and drying at 70° and 1 mm. (m.p. 166.5–170°).

Anal. Calcd. for $C_{18}H_{17}N_7O_{10}$: C, 46.67; H, 3.70; N, 15.12. Found: C, 46.82; H, 3.98; N, 15.29.

The analysis corresponds to that expected from the picric acid salt of acetylated hydroxycotinine.

Concentration of the eluates in 11–50% methanolic ether yielded 150 mg. of colorless crystals. For analysis these were recrystallized from benzene, m.p. 135–136°, and dried at 70° under diminished pressure, $[\alpha]^{25}_{5461}$ −60.7° (*c* 1.65, methanol).

Anal. Calcd. for $C_9H_{10}N_2O$: C, 66.65; H, 6.22; N, 17.27. Found: C, 66.34; H, 6.31; N, 17.3.

Synthetic (−)-desmethylcotinine did not depress the melting point of the compound and showed the same R_f values upon chromatography on paper.

(−)-Nicotine from (−)-Cotinine.—A solution of cotinine (4.5 g.) in 100 ml. of dry tetrahydrofuran was added dropwise to 90 ml. of tetrahydrofuran containing 1.2 g. of lithium aluminum hydride at 0°. After refluxing for 40 hr., the solution was treated with 70 ml. of acetone[22] and then with 100 ml. of 5 *N* NaOH. The aqueous phase was separated and washed twice with chloroform. The combined organic phases were dried over anhydrous sodium sulfate and concentrated to 5.5 g. of light-colored oil. The oil was dissolved in chloroform and placed on a column of acid-washed alumina (200 g.). By elution with ether containing successively increasing amounts of methanol, two Koenig positive fractions were obtained. The minor fraction, obtained last from the column, consisted of 0.31 g. of oil, R_f 0.89 (ammonia–ethanol–butanol). Nicotine, the major fraction, (2.3 g.) (R_f 0.84), was converted in methanol to the dipicrate, m.p. 223–224.5° (4.9 g.). The mixed melting point with authentic material was not depressed. The purified dipicrate (2.37 g.) was treated with 0.5 *N* HCl (50 ml.). After removal of picric acid by filtration, the aqueous phase was made alkaline with ammonia and extracted with chloroform. The chloroform extracts were concentrated to an oil. The latter was dissolved in methanol and treated with decolorizing carbon. The filtrate was concentrated in a stream of nitrogen to yield 0.38 g. of nicotine (dried under diminished pressure), $[\alpha]^{25}_{5461}$ −162.5° (*c* 2.75, acetone).

The *dl*-Desmethylcotinine.—γ-(3-Pyridyl)-γ-oxobutyric acid[11,23] (15 g.) was hydrogenated at 80 atmospheres and approximately 130° in 300 ml. of absolute ethanol containing 30 g. of ammonia and approximately 5 g. of Raney nickel for 7 hr. The filtrate from the catalyst was concentrated to an oil. The latter was dissolved in dilute ammonia water. An extraction with three portions of benzene (150 ml. each) served to remove some red colored material and facilitated a final extraction with five portions of chloroform (150 ml. each). After drying over anhydrous sodium sulfate the chloroform extract was concentrated to 10.2 g. of oil which

(17) E. B. Keller, J. R. Rachele and V. du Vigneaud, *J. Biol. Chem.*, **177**, 733 (1949).

(18) E. Werle, H. Schievelbein and D. Spieth, *Arzneimittel-Forsch.*, **6**, 322 (1956).

(19) S. Sugawawa, T. Tatsuno and T. Kamiya, *Pharm. Bull. (Japan)*, **2** [1], 39 (1954).

(20) Microanalyses by Weiler and Strauss and Spang Microanalytical Laboratory.

(21) Q. McKennis, E. R. Bowman and H. McKennis, Jr., *Toxicol. Appl. Pharmacol.*, **1**, 61 (1959).

(22) H. McKennis, Jr., and G. W. Gaffney, *J. Biol. Chem.*, **175**, 217 (1948).

(23) R. N. Castle and A. Burger, *J. Amer. Pharm. Assn. Sci. Ed.*, **43**, 163 (1954). These authors reported that they were unable to convert the keto acid into desmethylcotinine by catalytic reduction in the presence of ammonia.

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solidified on standing. The latter was recrystallized twice from benzene to give 7.2 g. of desmethylcotinine monohydrate, m.p. 65–68°. Admixture with an authentic sample[11] produced no melting point depression. The product at this stage is sufficiently pure for resolution. Further purification may be effected by chromatography of a benzene solution on alumina and then elution with 5–90% methanolic ether. The fractions from methanolic ether (20–90%) yield a crystalline form from benzene, m.p. 89–91°, which differs in melting point from the previously described[11] anhydrous or monohydrated compound. Upon standing in the open air the compound in a few hours forms a monohydrate which melts at 66–67°.

(−)-Desmethylcotinine.—*dl*-Desmethylcotinine (1.80 g.) and 2.50 g. of *d*-10-camphorsulfonic acid were dissolved in 200 ml. of warm ethanol. The cooled solution was treated with 100 ml. of ether and placed in an ice-bath. Crude (−)desmethylcotinine *d*-10-camphorsulfonate precipitated and was separated from mother liquor A. Recrystallization from 100 ml. of ethanol yielded 1.33 g. of the purified salt, m.p. 222–225°, and mother liquor A-1. A solution of the salt in 5 ml. of water was made alkaline with ammonia and then extracted with five portions of chloroform (7 ml. each). The combined chloroform extracts were dried over anhydrous sodium sulfate and then evaporated to yield colorless crystals (0.35 g.). These melted at 135–137.5° after one recrystallization from benzene (0.31 g.), $[\alpha]^{25}D$ −60.9° (*c* 1.12, methanol), $[\alpha]^{25.5}_{5461}$ −60.5° (*c* 2.81, methanol).

(+)-Desmethylcotinine.—The mother liquor A from the crude *d*-camphorsulfonic acid salt of (−)-desmethylcotinine (corresponding to 10.8 g. of *dl*-desmethylcotinine) was concentrated to dryness to give 16.6 g. of crude *d*-camphorsulfonic acid salt of (+)-desmethylcotinine. To a solution of the salt in 200 ml. of ethanol was added 100 ml. of ether. Upon cooling in the ice-bath 9.1 g. of salt was deposited. The latter was decomposed with ammonia water and 3.03 g. of crude (+)-desmethylcotinine (m.p. 68–110°) was deposited. The latter (2.51 g.) was dissolved in 140 ml. of absolute ethanol and treated with 3.58 g. of *d*-camphorsulfonic acid. Upon cooling to 0° the solution yielded 5.0 g. of salt and mother liquor B. The salt was combined with another lot obtained from evaporation of mother liquor A-1 (from recrystallization of *d*-10-camphorsulfonic acid salt of (−)-desmethylcotinine above). The combined lot was dissolved in 300 ml. of ethanol The solution deposited crystals upon cooling in the ice-bath. This crop then was recrystallized from 400 ml. of ethanol and finally from 200 ml. of ethanol to give 1.5 g. of optically pure *d*-10-camphorsulfonic acid salt of (−)-desmethylcotinine, m.p. 227–228°, and combined mother liquors C. The salt gave 0.3 g. of (−)-desmethylcotinine, m.p. 134.5–136°.

Mother liquor B was treated with 300 ml. of ether. Upon standing overnight in the refrigerator the solution yielded 0.45 g. of salt, m.p. 196–198°. The latter upon treatment with ammonia and chloroform gave (+)-desmethylcotinine, 80 mg. after recrystallization from benzene, m.p. 133–135°, $[\alpha]^{25}D$ +60.3° (*c* 1.16, methanol).

Mother liquor C upon evaporation to dryness gave 13.0 g. of salt. This was dissolved in 400 ml. of ethanol and then cooled in the ice-bath. Crystals, 5.37 g., were collected. The filtrate was treated with 400 ml. of ether and cooled to 0°. Salt (5.25 g., m.p. 193–210°) was collected and decomposed to give 2.06 g. of crude (+)-desmethylcotinine. Recrystallization from benzene afforded 1.0 g. of (+)-desmethylcotinine, m.p. 133–137°.

γ-(3-Pyridyl)-γ-*N*-methylbenzamidobutyric Acid.—A solution of 15 g. of γ-(3-pyridyl)-γ-methylaminobutyric acid monohydrate and 2.9 g. of sodium hydroxide in 70 ml. of water was treated (always keeping the *p*H above 9) with a total of 410 ml. of 10% sodium hydroxide and 72 g. of benzoyl chloride in alternate portions with cooling and shaking. The mixture was diluted to one l. and acidified with 2 *N* HCl (200 ml.). After removal of benzoic acid by ether extraction, the solution yielded the benzoyl derivative by direct precipitation at *p*H 6. The yield was 10.85 g. (50.9%), m.p. 173–175°. The mother liquor contains cotinine, unreacted methylamino acid and a small additional amount of the benzoyl derivative. For analysis a sample was recrystallized from ethanol, m.p. 175.5–176.5°, $[\alpha]^{26}_{5461}$ −127.8° (*c* 5, methanol).

Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.57; H, 5.96; N, 9.17.

Lamberts and Byerrum[28] prepared the benzoyl derivative by a similar procedure in 25% yield, m.p. 170°.

Dibromocotinine Hydrobromide Perbromide.—To a solution of 88 ml. (0.538 mole) of (−)-nicotine (Black Leaf "99") in 400 ml. of 80% acetic acid, 198 ml. of technical bromine (5.76 mole) in 480 ml. of 80% acetic acid was added dropwise with stirring over a 3.5 hr. period. 1800 ml. of water then was added to the orange solution containing a dark oily bottom layer. The solution was heated to approximately 80° until the oil had become miscible with the solvent. Upon cooling to room temperature the solution deposited orange needles. The solid was removed by filtration and washed with water. On drying in air the material, m.p. 140–145°, weighed 163 g. (70%, based on the empirical formula $C_{10}H_9N_2OBr_2 \cdot HBr \cdot Br_2$). On several successive runs the yields ranged between 60 and 75%. In contrast a yield of 39% was obtained by following the method of Pinner.[24]

(−)-Cotinine.—To 2500 ml. of 50% acetic acid 200 g. (0.34 mole) of dibromocotinine hydrobromide perbromide was added. After the addition of 125 ml. of concentrated HCl, 250 g. of zinc dust was added portionwise with stirring over a 3-hr. period. The solution was stirred for an additional hour and then, after removal of unreacted zinc, adjusted to *p*H 8–9 with concentrated ammonia water.[25] On cooling the alkaline solution was extracted three times with one l. portions of chloroform. Upon evaporation of the chloroform the residue spontaneously crystallized to give 56.4 g. of cotinine which had a boiling point of 145–150° under 0.7 mm. pressure. Lamberts and Byerrum reported a boiling point of 170–175° under approximately 1 mm. pressure. Cotinine obtained above or prepared by the method of Pinner was found usually to be chromatographically pure as disclosed by paper chromatography on Whatman No. 1 paper in the ammonia–butanol–ethanol and formic acid–*sec*-butyl alcohol–water systems.[26] However, certain samples of cotinine were found to contain an impurity at R_f 0.61 (cotinine R_f 0.75) as disclosed[27] in the butanol (100 ml.)–water (20 ml.)–ethyl *p*-aminobenzoate (0.3 g.) system[29] and also at R_f 0.61 in the ammonia–butanol–ethanol systems. Distillation of these samples at the temperature and pressure reported above failed to remove the impurity. However, this can be separated from the cotinine by alumina chromatography using methanol–ether as the eluting solvent.

RICHMOND, VIRGINIA

(24) A. Pinner, *Ber.*, **26**, 2807 (1893).

(25) Suggested by Mr. H. N. Wingfield, Jr., The American Tobacco Company, Research Laboratory.

(26) W. Hausmann, THIS JOURNAL, **74**, 3181 (1952).

(27) R. N. Jeffrey and W. H. Eoff, *Anal. Chem.*, **27**, 1903 (1955).

(28) B. L. Lamberts and R. U. Byerrum, *J. Biol. Chem.*, **233**, 939 (1958).

(29) Dr. R. A. Clayton of The American Tobacco Company, Research Laboratory, kindly called our attention to the procedure using butanol–water–ethyl *p*-aminobenzoate.

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TOBACCO INDUSTRY RESEARCH COMMITTEE

#255

150 East Forty Second Street

New York 17, N.Y.

Dr. Bing, Chm.
Dr. Cattell
Dr. Comroe
Dr. Rienhoff

Application For Research Grant

Date: October 25, 1959

1. Name of Investigator: Victor A. McKusick, M.D.

2. Title: Associate Professor of Medicine - Physician-in-charge, Joseph Earle Moore Clinic
Assistant Professor of Epidemiology - Chief, Division of Medical Genetics

3. Institution & Address: Johns Hopkins University School of Medicine
Baltimore 5, Maryland

4. Project or Subject: A combined genetic, physiologic, clinical, and epidemiologic study of Buerger's disease.

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5. Detailed Plan of Procedure:

Objectives: To seek answers to the following questions:

a. Is Buerger's thromboangiitis obliterans a distinct entity?
b. Is there an important genetic factor in its etiology?

By-products will include an evaluation of present frequency of the disease in relation to that in the past, and an analysis of the clinical and histopathologic pictures.

Method of study: Ascertainment, criteria and controls.

The general plan is to characterize the families of cases of Buerger's disease. If Buerger's disease is merely a variant of ordinary atherosclerosis, then a concentration of atherosclerotic complications should be observed in such families, as well as other features such as statistically higher serum cholesterol valves. If members of the family show peculiarities, as compared with controls, such as greater physiologic sensitivity to tobacco and nicotine (changes observed in Buerger's cases themselves), then evidence suggesting a familial factor in the etiology of the disease is provided.

Despite the seeming rarity of new cases, material for a family study should be adequate since relatives of cases accumulated over the last 10 or 15 years in most Baltimore hospitals will be used.

Probands (index cases) will be ascertained by surveying cases diagnosed Buerger's disease in Baltimore hospitals. Only cases with onset before the age of 40 years will be selected; cases with diabetes will be excluded.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Controls will be clean surgical cases (hernia, appendectomy, fractures, etc.) admitted to the same hospital in the same calendar year and matched for age, sex and race.

In addition to a clinical analysis of the proband cases and a review of the histopathology* (in collaboration with Dr. Sumner Wood of the Department of Pathology) the following studies will be performed in members of the families (first degree relatives - parents, sibs, children) and in probands when available:

1. Serum cholesterol (facilities for triglyceride determination will probably also be available).

2. Physiologic response to cigarette smoking and nicotine administration, as measured by pulse rate, blood pressure, electrocardiogram, skin temperature and finger-tip plethysmogram. (Dr. J. Edwin Wood has visited us and advised us in the design of plethysmographic studies). Dr. Frank B. Davis has offered to study ballistocardiographic responses in the same subjects.
In the cases themselves and their controls an epidemiologic questionnaire to get information on religion, tobacco history, occupational history, residential history, exposure to the elements, e.g., temperature extremes, and a variety of other factors, will be executed. Furthermore, a total clinical evaluation of the probands will be performed.
In the families a complete medical evaluation with particular emphasis on cardiovascular disease will be performed with collection of death certificates and all available records from physicians and hospitals.

6. Budget Plan: (Budget for 18-month period)

Salaries (research asst. $3,000 per annum)	$4,500. (S.S.incl.)
Expendable Supplies	1,500.
Permanent Equipment (plethysmograph)	1,500.
Overhead (15%)	1,200.
Other (travel, etc., in family studies)	500.
Total	$9,200.

7. Anticipated Duration of Work: 18 months.

8. Facilities and Staff Available:
   a. An air conditioned room with satisfactorily constant temperature (tested by Dr. Wood in connection with venous occlusion plethysmography).
   b. Two research fellows: Dr. Ralph Weber
      Dr. John M. Levitsky

9. Additional Requirements: -----

* A considerable amount of pathologic material is available, mainly in the form of amputated limbs but also in a few autopsies.

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Source: https://www.industrydocuments.ucsf.edu/docs/thhd0000

10. Additional Information (Including relation of work to other projects and other sources of supply):

The proposed research will be conducted in the Joseph Earle Moore Clinic and the Division of Medical Genetics of the Department of Medicine. Dr. A. M. Lilienfeld, Associate Professor of Medicine and Associate Physician-in-charge of the Moore Clinic is advising us in the epidemiologic aspects of the study, including the selection of controls. Genetic factors in cardiovascular diseases is a major research interest of the Division of Medical Genetics. The two research fellows mentioned above are supported from other sources. No other financial support for this specific project is available.

/s/ Victor A. McKusick, Director of Project

/s/ Thomas B. Turner, Business Officer

10035441932

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: October 11th, 1954

1. Name of Investigator:

John T. McLaughlin, M.D.

2. Title:

Director of Biophysics Research

3. Institution & Address:

Institute of Nuclear and Atomic Sciences
106 Chestnut Street, Burbank, California

4. Project or Subject:

Analysis of Tobacco for radioactivity...a quantitative and qualitative determination by means of mass spectroscopy.

A preliminary survey to determine the feasibility, mechanics and cost of such analyses.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Mass spectroscopy is a proved and accurate method of chemical analysis. We know that there are radioactive isotopes present in tobacco (see Paragraph 10). Now we must determine what they are and in what amounts. Mass spectroscopy is apparently a faster and more accurate method of determination than the current chemical and physical methods. In fact, the chemical isolation of the isotopes is a monumental task. Since mass spectroscopy in solids is a new development done in only a few laboratories it behooves us to survey the problem before attempting to estimate the time and cost of such studies. From the practical viewpoint we must determine if these studies can be done in present facilities on a contract basis, or we should build our own instrument and operate it solely for this study. The people currently doing such analyses have agreed to a trial survey and this application is for money for such a trial in order to determine, practically, how to proceed further.

Therefore, the plan of procedure is to go to the institute where mass spectroscopy on solids is being done and proceed with an analysis x of tobacco to determine qualitatively the isotopes present which are radioactive. After this is accomplished an additional application will be made in the light of the knowledge gained.

Yet is radioactivity organic or inorganic ? Foods are also radioactive Inorganic + prob. is silica to bones + can be controlled but not only half life but duration in time & accumulated & excreted as well. Also are expected percent of radiation? Can be always certain each constituents Amazing Lebanese could do all studies for.

1003541633

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

if it stays there

smoking at the above rate the tumorgenic dose is being exceeded. These figures must be corrected for half-life when the isotope is isolated, but our studies indicate it to be a hard beta emittor. The possible isotopes to be found include those of long and short half-life, but in a cigarette after picking and storing the tobacco, processing the cigarette and then storing these, the short half-life isotopes should be exhausted for practical purposes. Therefore, in our studies we are looking for a long-life hard beta emitting isotope. After the isotope is isolated and animal studies are accomplished then a correction can be made for the biological half-life.

FURTHER STUDIES

These should be done step by step and application made for each successive step.

I. Identification and quantitative study of radioactive isotopes contained in tobacco, tobacco ash and smoke.

A. This can probably be done faster, more accurately and cheaper by mass spectroscopy than by any other means. Many samples and strains should be studied to see if this is a constant or a variable factor.

B. If the etiology of the isotopes is not self-evident from the above studies, then separate analyses should be made on the soil, water, fertilizer and air from the area where the tobacco is grown. Similar studies should be done on cigarette paper and any other ingredients of cigarettes.

II. Attempt to isolate offending isotope from lungs invaded by neoplasia.

III. Produce tumors in appropriate animals with this isotope and study the metabolism of this material.

IV. Study of feasible methods of prophylaxix and/or decontamination. In this category one must consider the health and welfare of personnel processing such tobacco.

1003541634

6. Budget Plan:

Salaries	John T. McLaughlin, M.D.	$1,000.00
Expendable Supplies		
Permanent Equipment		
Overhead	@10%	~~194.50~~
Other	Cost of preliminary study at contract laboratory: xxxx	~~500.00~~
	Travel Expense	445.00
	Total:	~~2,139.50~~ 2,139.50

7. Anticipated Duration of Work:

Two months.

8. Facilities and Staff Available:

None required other than John T. McLaughlin, M.D. and his personal clerical assistance.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

This preliminary survey should be accomplished before proceeding by steps with the further studies as here outlined. Realization of the problems of mass spectroscopy on solid material with an instrument sufficient to give a degree of resolution that will isolate and identify isotopes reveals the rationale of such a survey.

MAJOR THESIS

Preliminary studies in our laboratories on smoke from one completely burned cigarette revealed a radiation level of 3.75 mr/week. This is more than the figure chieved at the University of Leeds, and we feel that our smoke was contaminated by ash to a small extent. This is an accumulative figure since when smoke is inhaled and then exhaled about 12% of the solid material will remain in the lungs and/or bronchial tree. The average smoker consumes one package of twenty cigarettes/day; therefore, after one week he is exposed to 54 mr/week. Conceding the tumogenic dose of internal radiation (the figure on lung is still undetermined) to be in the vicinity of 30,000 mr/week, it is possible that after ~~five or six~~ 55 weeks of

always will be

Signature ~~Director of Project~~ John T. McLaughlin, M.D.

Kenneth A. Smith, DSc.
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tjvd0000

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant #162

Date: May 20, 1957

1. Name of Investigator: Jack Neal

2. Title: Associate Professor and Director of Industrial Hygiene Research Laboratory

3. Institution & Address: Department of Preventive Medicine & Public Health
University of Texas - Medical Branch
Galveston, Texas

4. Project or Subject:

Analysis of tars collected from cigarettes, manufactured from a single variety of tobacco, using smoking methods comparable to the average smoker.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

1. Obtain cigarettes manufactured from the four most widely used varieties of tobacco.
2. Use Phipps - Bird smoking machine, puffing 35cc of air at 58 second interval, for a 2 second duration.
3. Redistilled methanol to be used as the collection medium.
4. Use powder chromatography to separate the hydrocarbons in the collected tar.
5. Weigh all compounds separated to 0.01 grams sensitivity.
6. Compare each fraction, separated from the different varieties, using the double beam infrared spectrophotometer with silver chloride plates and potassium bromide pellets.

1003541637

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	$ 6,180.00*
Expendable Supplies	1,000.00
Permanent Equipment	1,000.00
Overhead (15%)	1,227.00
Other	
Total	9,407.00

*Includes Social Security and Workmen's Compensation Insurance.

7. Anticipated Duration of Work:

One Year

8. Facilities and Staff Available:

See attached sheet

9. Additional Requirements:

1. Personnel
   A. One laboratory assistant
   B. One laboratory helper
2. Equipment and supplies
   A. Chromotography supplies, powder, glassware, chemicals, etc.
   B. Cigarettes manufactured similar to those commercially available, but composed of a single variety of tobacco.

10. Additional Information (including relation of work to other projects and other sources of supply):

A. Relationship to other work:

1. The applicant has been engaged in a similar project since 1953 in which separation and identification work has been done on the commercially available cigarettes smoked under standard conditions. Also animal work has been done on the separated compounds of the tars recovered from the smoking apparatus. As a result of these four years of work a procedure has been worked out for the separation and identification of some of the principal chemical compounds found in tars collected under standard smoking conditions and the applicant wishes to apply this procedure to cigarettes composed of individual varieties of tobacco. It is the opinion of the applicant that if this procedure is applied to individual varieties of tobaccos the cigarettes must be made under the same conditions that the commercially available ones are made to obtain uniform results. Therefore, it is necessary to apply for a grant of money to defray the expense of securing these cigarettes.

Signature Jack Neal
Director of Project

E. D. Walker
Business Officer of the Institution

100354163 8

Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

8. Facilities and Staff Available:

A. Facilities

1. Space

Two large fully equipped chemical laboratories about 1200 ft. of floor space. These laboratories are especially designed for industrial toxicology and research.

2. Equipment

1. Double beam Baird infrared spectrophotometer.
2. Single beam Beckman infrared spectrophotometer.
3. D. U. Beckman spectrophotometer.
4. Hilger medium quartz spectrograph and plate reader.
5. Extra large Spencer polarizing microscope with complete accessories.
6. Phipps - Bird smoking apparatus.
7. 250 cigarette model universal smoking apparatus.
8. Complete powder chromotographic equipment.

B. Staff

1. Professor Jack Neal, director of Industrial Medicine Laboratory.
2. Available consultants in the fields of biochemistry, pharmocology and industrial medicine.
3. Laboratory assistants.

1003541639

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

#245

150 East Forty Second Street

New York 17, N.Y.

Date: July 21, 1959

1. Name of Investigator: Jack C. Norris, M.D.

2. Title: Physician

3. Institution & Address: Norris Clinical Lab.
490 Peachtree Street, Suite 138
N.E. Atlanta, Ga. 8

4. Project or Subject: Investigation of the effect smoking tobacco may or may not have on the common cellular components of human blood.

5. Detailed Plan of Procedure: It is proposed that in our laboratory, where we see a number of patients representing a fairly good cross section of our community, to try and determine by ordinary blood counts, stains, etc. whether the blood is affected: leucocytes, lymphocytes, eosinophiles, erythrocytes and platelets: are they depressed, increased, or changed in any manner that we can determine.

Likewise each patient would be checked as to name, age, sex, genetic background; cigarettes smoked, how long, brand, filtered or not; history of cancer in families, especially cancer of the lung.

After a suitable period of time the charts would be carefully reviewed and analyzed. If the study shows anything of importance, then a further request for extension of the stipend will be requested.

If possible, in due time, these cases should be followed to see if cancer has developed in any of the patients; however, that part of the project should be done in a statistical division of the Committee.

The primary study here related, should probably last for approximately 24 months. The money therefrom to be used to defray expenses, salaries, etc. incident thereto.

1003541640

6. Budget Plan:

Salaries	$100.00
Expendable Supplies	25.00
Permanent Equipment	25.00
Overhead	25.00
Other	25.00
	200.00 per mo.
Total year	$2,400.00

7. Anticipated Duration of Work: 24 months.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

8. Facilities and Staff Available:

Myself, and two assistant technicians

9. Additional Requirements: Cannot say at this time.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Cannot answer at this time.

/s/ Jack C. Norris, M.D.
Director of Project

- above -
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: April 19, 1956

1. Name of Investigator: Adrian M. Ostfeld, M.D.

2. Title: Assistant Professor of Preventive Medicine

3. Institution & Address: University of Illinois College of Medicine
840 South Wood Street
Chicago, Illinois

4. Project or Subject: A biomicroscopic assay of bulbar conjunctival vascular reactivity in hypertension employing topical and parenteral pharmacologic agents.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The arterioles, capillaries and venules of the bulbar conjunctival vessels may be readily examined by means of a Poser Ophthalmic slit lamp at a magnification of 47.5X and appropriate photographs made. 1 The effects of pharmacologic agents may be assayed either by preparing them in an isotonic phosphate buffer at a pH of 7.4 and applying them directly on the bulbar conjunctivae or after parenteral administration. When one eye is treated and the other serves as control, transient changes in systemic hemodynamics and variations in absorption rates of different agents do not prejudice experimental results. These technics will be employed in the study of the following problems.

The studies of Goldenberg on blood pressure responses [2], Lee on bulbar conjunctival minute vessels [3], and Griesman [4] on nail fold capillaries have all concurred in the observation that small blood vessels of hypertensive patients are more reactive to epinephrine and nor-epinephrine than those of normotensives. Since the latter agent is probably the true sympathetic vasoconstrictor substance, hyper-reactivity to nor-epinephrine may play an important role in the genesis and maintenance of the hypertensive state.

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Detailed Plan of Procedure - Cont'd.

Such hyperreactivity in hypertension may be determined by one or more of the following mechanisms: 1) an hydraulic and mechanical effect; i.e. a vessel already partly constricted may more readily constrict further with blood viscosity assuming greater importance in small diameter tubes, 2) the presence of a circulating agent or agents which potentiates nor-epinephrine effects 3) a true vascular smooth muscle hyperreactivity to nor-epinephrine.

The following proposed studies would help to determine which of the above mechanisms is relevant. 1) In hypertensive and normotensive subjects, the diameter of representative arterioles will be measured by micrometer and the threshold to topical nor-epinephrine determined. (Threshold is defined as the least concentration of nor-epinephrine which predictably closes metarteriolar sphincters and blanches capillaries.) The arterioles would then be doubled in diameter by means of topical applications on separate occasions of three structurally and chemically unrelated vasodilators; histamine, acetylcholine combined with pilocarpine, isopropyl nor-epinephrine. If vasodilation decreases nor-epinephrine reactivity (i.e. raises threshold) the inference is that arteriolar diameter per se, and therefore mechanical and hydraulic factors largely determine this vascular response. If diameter changes do not alter reactivity, the other possible mechanisms thereby receive support.

2) In both hypertensive and normotensive subjects, the effects of other topically applied vasoconstrictors, namely nicotine and pitressin would be studied. If there is hyperreactivity to these agents in hypertensive subjects or if nor-epinephrine thresholds are lowered by pre-treatment with these vasoconstrictors, then the effects of nor-epinephrine are not specific and again a mechanical and hydraulic factor appears most relevant. Again, if the converse is true, the other theses become more likely.

3) An opportunity is herein afforded to study the effects of topical and injected nicotine on the minute vessel appearance and reactivity in normotensive and in hypertensive subjects and to assess a possible relevance of nicotine (and less directly of smoking) in the genesis and progression of hypertensive vascular disease.

4) The vascular appearance and reactivity of the bulbar conjunctivae in normal subjects transiently made hypertensive by intravenous nor-epinephrine will be studied, and a comparison made with the same vascular properties in hypertensive subjects. If no differences are manifest then it is highly unlikely that the vascular smooth muscle behaves differently in hypertensive subjects.

While none of these experiments by itself would serve conclusively to establish the mechanism of nor-epinephrine hyperreactivity in hypertension, reliable evidence to support one or another mechanism would be obtained.

(see next page for references)
page three

10034194

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

1. Ostfeld, et. al., HEADACHE AND HYDRATION, A.M.A. Archives of Internal Medicine, August 1955, Vol. 96, pp. 142-152

2. Goldenberg, et. al., HEMODYNAMIC RESPONSE OF MAN TO NOR-EPINEPHRINE AND EPINEPHRINE AND ITS RELATION TO THE PROBLEM OF HYPERTENSION, Am. J. Med., Vol. 5, p. 792, 1948

3. Lee and Holze, PERIPHERAL VASCULAR HEMODYNAMICS IN THE BULBAR CONJUNCTIVA OF SUBJECTS WITH HYPERTENSIVE VASCULAR DISEASE, The Journal of Clinical Investigation, Vol. XXX, No. 6, pp. 539-546, June, 1951

4. Greisman, THE REACTION OF THE CAPILLARY BED OF THE NAILFOLD TO CONTINUOUS INTRAVENOUS INFUSION OF LEVO-NOR-EPINEPHRINE IN PATIENTS WITH NORMAL BLOOD PRESSURE AND WITH ESSENTIAL HYPERTENSION, The Journal of Clinical Investigation, Vol. XXXIII, p. 975, July, 1954.

5. Von Euler, THE NATURE OF ADRENERGIC NERVE MEDIATORS, Pharm. Rev., Sept. 1951, p. 247.

100354645

Source: https://www.industrydocuments.ucsf.edu/docs/xmcb0000

6. Budget Plan:

Salaries	$4000.00
Expendable Supplies	500.00
Permanent Equipment	500.00
Overhead 12%	1300.00
Other	0.00
Total	$6300.00

7. Anticipated Duration of Work: 2 years

8. Facilities and Staff Available: 1. A research bed in the Research and Education Hospital affiliated with the Medical School. 2. The patients load and facilities of the Hypertension Clinic and other OPD Clinics of the hospital. 3. A laboratory in the hospital prepared in anticipation of this study. 4. Adrian M. Ostfeld, M.D. Assistant Professor of Preventive Medicine. 5. A technician and assistance from the resident level medical house staff.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

No other sources of supply.
This project grew out of a two-year study of bulbar conjunctival vascular reactivity in headache in the laboratories of Dr. Harold G. Wolff, Professor of Medicine (Neurology), New York Hospital-Cornell Medical Center.

100541646

/s/
Signature Director of Project Adrian M. Ostfeld

Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/fmnb0110

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APR 26 1956
PHILIP MORRIS & CO. LTD. INC.
RESEARCH & DEVELOPMENT DEPT.

1003541647

a. Budget Plan:

Salaries	$ 24,000.00
Expendable supplies	2,000.00
Permanent equipment	500.00
Overhead	2,000.00
Other	0.0
Total	$ 28,500.00

7. Anticipated Duration of Work: 3 years

8. Facilities and Aid Available: A. Is research now being carried on in the Research Department of your institution which is related to the proposed project? If so, name the individuals engaged in such research and describe briefly the facilities available.

B. Additional Requirements: None

(Supply additional information relative to work of other projects now under way in your laboratory which may duplicate the proposed project.)

All orders, contracts or subcontracts let by the Agricultural Research Service in connection with this project will be subject to the usual terms and conditions of the Department of Agriculture. Payments under such orders, contracts or subcontracts will be made only on the basis of actual costs incurred in the performance thereof.

Director of project

Business Officer of the institution

1003541648

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#134CR1

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

(Activated 3/1/53)

Date: November 11, 1953

1. Name of Investigator: Donald M. Pace, Ph.D.

2. Title: Professor of Physiology

3. Institution & Address: University of Nebraska
Lincoln, Nebraska

4. Project or Subject: A continuation of investigations into the effects of certain constituents of tobacco smoke on tissue cells cultivated in vitro.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The same procedure as given in previous application will be followed. However, more attention will be given to long range effects of selected substances in concentrations that appear to have no effect over short periods of time.

1003541649

Source: https://www.industrydocuments.ucsf.edu/docs/sfty0000

6. Budget Plan:

Salaries	~~8000.00~~ (6500.) (1500.)
Expendable Supplies	~~1000.00~~
Permanent Equipment	
Overhead	
Other	~~750.00~~
Total	~~9750.00~~

7. Anticipated Duration of Work: 1 year

8. Facilities and Staff Available: The Institute for Cellular Research consists of a suite of 8 rooms well equipped for short and long term tissue culture investigations. Although, the above request is made for the support of one investigator, actually all the staff members of the Institute enter into some phase of the work.

9. Additional Requirements: We are using a synthetic nutrient medium in which to cultivate our cells. Many of the chemicals used in this medium are very expensive and this is the reason for a slight increase in our request for funds for expendible supplies along with the fact that we will need more culture flasks.

10. Additional Information (Including relation of work to other projects and other sources of supply):

As stated in a previous request, we are still working on a project designed to give information concerning the effects of air pollutants on tissue cells cultivated in vitro. We are at present studying the effects of hydrocarbons, ozone and oxonides.

100354650

Signature _____
Director of Project Donald M. Face

_____
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/hfnb0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #134C

Report No. 1

Donald M. Pace, Ph.D.
University of Nebraska

March 1, 1959

Studies of the Effects of Some of the Constituents of Tobacco Smoke on Various Strains of Tissue Cells Cultivated In Vitro.

Rather extensive studies of the effects of two tobacco smoke constituents are nearing completion. Acetaldehyde and acetone were selected primarily because of their relatively high quantity, in comparison to others, in the gaseous phase of tobacco smoke. According to analyses, both of these two substances occur in the quantity of two milligrams per smoke of one cigarette.

Both short term experiments involving exposure times of less than one month and long term experiments, the so called time-concentration experiments, involving approximately six months of exposure have been conducted.

Four cell strains have been used in investigating these substances. They are Earle's strain L cells, Gey's HeLa cells, Mouse liver epithelial cells and Human skin cells. Stock cultures of these cells were maintained in T-60 flasks, at a constant temperature of 37.5°C in Earle's NCTC #109 medium supplemented with 10% horse serum. The medium was changed three times a week. Subculturing of the Liver cells and L cells was done routinely every week. In the cases of Skin cells and HeLa cells subculturing was frequently done as often as every three or four days. New and young T-60 cultures were gassed with a filtered 5% carbon dioxide and air mixture. The gassing procedure was used to assist in maintaining proper pH of the medium since it utilizes a carbonate buffer.

Checks on the toxicity and possible influence of these substances upon rate of proliferation were made periodically by the replicate method of culture. The replicate cultures were maintained at a constant temperature of 37.5°C, and received changes of appropriate medium three times a week. The experimental cells were usually exposed to the medium containing either acetaldehyde or acetone 48 hours after replication. Gassing of the Carrel cultures was done at every medium change throughout the experiments.

Two or 3 times a week 3 Carrel flasks from each group of control and experimental cultures were selected at random and sacrificed for enumeration. The medium of the cultures selected for counts was discarded and a known quantity of 0.1M solution of citric acid was added to each culture. They were placed in the incubator at 37.5°C for 2 to 3 hours. Nuclei counts were made by means of the Coulter Electronic Counter.

Data were accumulated in the form of recorded microscopical observations of the general appearance of the cells, photomicrographs of the cultures, and population growth curves.

1003541651

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Future plans call for investigating the effects of other individual constituents of tobacco smoke, whole tobacco smoke and some of the tar fractions on cell cultures in vitro. In some instances, when deemed advisable, time-concentration experiments will be carried out. Tentatively it is planned that some of the cell cultures from the time-concentration experiments will be used in injection experiments, involving mice, to determine if possible the acquisition of tumorgenic capacities by the cells.

A summary of the investigations of the short term experiments and the first 2 series of the acetaldehyde time-concentration experiments which were conducted, is presented here. Results of the acetone short term experiments and the final series of the acetaldehyde time-concentration experiments will be included in the next progress report.

ACETALDEHYDE

Short term experiments

All four cells types previously mentioned have been used in these investigations. Six different concentrations of acetaldehyde were used, two concentrations in each of three series: 8.0 mg and 2.0 mg per ml; 1.0 mg and 0.01 mg per ml; and 0.5 mg and 0.05 mg per ml. To prepare the experimental medium, the following procedure was used. The acetaldehyde, flasks, pipettes and regular medium were chilled. This was considered advisable because of the extremely low boiling point, 20°C, of acetaldehyde. Chilled acetaldehyde was quickly added to the cold medium and mixed thoroughly. Dilutions were made immediately. The cold medium was filtered, bottled in sterile 125 ml and 50 ml boiling flasks, tightly stoppered and stored under refrigeration. The medium was filtered this one time in order to minimize loss of acetaldehyde by vaporization.

Cells from 7 to 10 day old replicate cultures in Earle's NCTC #109 medium plus 10% horse serum were set up in Carrel D-3.5 flasks. After the cultures became well established, usually after 48 hours, they were exposed to the appropriate concentrations of acetaldehyde. Controls were maintained. Cell counts were made daily the first 3 and 4 days and from then on usually every other day. The usual techniques and precautions for handling such cultures were followed.

No conclusions were drawn from the short term experiments except in the cases where the concentrations were obviously toxic. The main objective of the short term experiments was to determine concentrations to be used in the long term or time-concentration experiments. The effects of the short term exposure are described in respect to morphological changes and populations.

1003541652

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Series 1. One experiment with concentrations of 8.0 mg and 2.0 mg acetaldehyde per ml of medium was made with each of L cells, Liver cells, and Skin cells. Morphological changes were soon visible in cells exposed to 8.0 mg per ml. The most conspicuous changes were the rounding up of the cells and subsequent detachment.

In the case of the cultures receiving 2.0 mg per ml, fewer cells were rounded and detached. In fact, to all outward appearances these cells continued to resemble the controls. Only upon observations of the resistance of cells to the citric acid preparatory to counting, rapid disintegration of the cells when returned to normal medium, and the use of time-lapse photography did not become apparent that these cells were dead and probably fixed.

Eight and 2 mg acetaldehyde per ml proved toxic to these cells in a matter of hours.

Series 2. Two experiments with concentrations of 1.0 and 0.01 mg of acetaldehyde per ml of medium were made with each in the case of Skin cells, HeLa cells and Liver cells, only one test was made with L cells.

The effects of 1.0 mg per ml were soon evident in that the cells rounded and detached; in the case of Liver cells within 1½ to 2 hours; in HeLa cells within 3½ hours.

1003541653

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

In respect to 0.01 mg per ml, no definite morphological alterations were recognized under ordinary microscopical examination. There were times, after a week or more of exposure, when it did appear as though the experimental cells were not as healthy as the controls. The population growth curves constructed from the cell counts suggested a slight inhibitory effect. Since only two tests were made and the time of exposure was relatively short, no conclusions were drawn. It was felt that the indication of an effect was sufficient to use this concentration in the time-concentration experiments. Series 3. One test with the concentrations of 0.5 and 0.05 mg acetaldehyde per ml of medium was made with each of the four cell strains.

Cells treated with the 0.5 mg per ml concentration began to round and detach within a few hours. As in the case of cells treated with 0.1 mg per ml detachment became extensive enough that the cultures were discarded within three to four days. It was interesting to note that cells treated with the 0.5 mg per ml, although rounded and detached, were not killed immediately. A single check on the percentage of survival was made on HeLa cells, Liver cells and L cells using erythrosin B stain. Erythrosin B stains dead cells quickly and by using the hemocytometer, counts were made by which percent of survival was determined. In the case of L cells, 48 to 96 hours exposure resulted in killing all cells; with HeLa cells, 50% were surviving at the end of 46 hours; all of the Liver cells succumbed between 25 hours and 48 hours exposure, the greatest rate of death being between 4 and 25 hours of exposure.

The results obtained with the cells exposed to 0.05 mg per ml were similar to those obtained with the concentration of 0.01 mg per ml. Morphological changes were not detected by the usual microscopical examinations. Population curves again suggested a slight inhibitory effect. The indication was felt sufficient to use this concentration also in the time-concentration studies.

1003541654

Time-Concentration Experiments

In these experiments three cell strains were employed, Mouse Liver cells, Earle's strain L cells, and HeLa cells. The two concentrations of acetaldehyde used were 0.01 and 0.05 mg per ml of medium.

Nine replicate cultures were set up in T-60 flasks by pooling 3 stock cultures of Liver cells. At the time of the second medium change (5 days later) the nine flasks were randomly divided into three groups. One of the groups was maintained on Earle's NCTC #109 medium plus 10% horse serum and carried as the control group. Another received the same medium plus 0.01 mg per ml of acetaldehyde. The third group was exposed to the same medium as group one plus 0.05 mg per ml of acetaldehyde. The same procedure was followed for L cells and HeLa cells.

All 27 cultures constituted the parent stock cultures of control and experimental cells for the entire experiment. They were kept at a constant temperature of 37.5°C. The medium was changed every 48 hours with the exception of the week-ends when the interval of time between changes was 72 hours. Every week the Liver cells and L cells were subcultured, while HeLa cells were often subcultured more frequently. Periodically, replicate cultures were begun in Carrel flasks with cells obtained from the controls, the 0.01 mg and the 0.05 mg cultures. Cell counts were made from time to time so that proliferation rates could be ascertained.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Series 1.

Mouse Liver cells:

Twenty-three days after the parent stock cultures had been established, replicate cultures were made in Carrel flasks with cells from the control and each of the two experimental groups. The initial inoculum for each set of Carrel flasks was slightly more than 300,000 cells per ml. These cultures were carried for 14 days. Population curves of the control and "0.01 mg cells" indicated rapid rates of proliferation which closely approximated each other. Contrary to the earlier impressions from short term experiments the 0.01 mg per ml. of acetaldehyde appeared to have no effect, or if any, a slight stimulatory effect. Microscopic observation of the control cells and the "0.01 mg cells" revealed healthy and flourishing cultures.

The "0.05 mg cells", although during the first day or two appeared normal, did not attach readily to the substratum and evidently did not proliferate. By the fourth day the "0.05 mg cultures" were sparse. Cells were detaching and there was inadequate pH adjustment of the medium and on the eighth day the cultures were discarded.

Earle's strain L cells:

After 40 days of exposure to the acetaldehyde treated medium, replicate cultures in Carrel flasks were made of the control and the two groups of experimental cells. The initial inoculum for the cultures was 500,000 cells per ml. These cultures were carried for 30 days. The cells of all three groups appeared healthy throughout the run and the cultures seemingly flourished. For a time, there appeared to be a slight inhibitory effect in the medium containing 0.05 mg per ml, however, the final populations in all three cases were approximately the same. Any harmful or beneficial effects, if existent, were negligible.

HeLa cells:

Replicate cultures were made 45 days after exposure to the acetaldehyde medium. Only the control cells and the "0.01 mg cells" were replicated, for at this time, difficulty was experienced in keeping the "0.05 mg cells" from sloughing extensively. The initial inoculum for both sets of cultures was approximately 350,000 cells. These cultures were maintained for 17 days. Microscopic examinations did not reveal any morphological differences between the two sets of cells. The population curves show comparable proliferation rates and comparable final populations.

Series 2.

1003541655

Mouse Liver cells:

A second series of replicate cultures of Liver cells were begun following 72 days of exposure. The initial inoculum varied somewhat among these sets. For the control cultures, the inoculum was approximately 600,000 cells per ml; for the "0.01 mg cells", approximately 550,000 cells per ml; and for the "0.05 mg cells", approximately 500,000 cells per ml. Part of the replicated "0.01 mg cultures" and "0.05 mg cultures" in Carrel flasks were returned to the regular control medium at the first medium change following replication.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

The "0.01 mg cells" and the "0.01 mg cells which had been returned to normal medium" appeared to proliferate extremely rapidly, and by the sixth day the number of cells had doubled in the former and more than doubled in the latter cultures as compared to the controls. Four days later, however, the cell number had dropped considerably below the number in the control cultures. After this, the increase in population was consistent in both though not quite reaching the maximum of the controls. The possibility appears that with such a rapid rate of proliferation at the beginning, the first maximum population for the "0.01 mg cells" and the "0.01 mg cells which had been returned to normal medium" (hereafter designated as "0.01 N cells") may have been reached. This overcrowding and eventual sloughing may have resulted within the intervening four days before the next count. The final maximum population may in reality have been a second maximum. These cells appeared to be healthy throughout the run.

The population curve for the controls appeared to be typical. These cells remained in fine condition and arrived at their maximum in 13 days.

The "0.05 mg cells" never became established in Carrel flasks hence were gradually lost in subsequent medium changes and had to be discarded. For the most part, they remained rounded and floating. The replicate cultures in T-60 flasks also had to be discarded after 89 days of exposure. The cells would not attach to the flasks. The "0.05 mg cells" which were returned to normal medium (hereafter designated as "0.05 N cells") after three days recovered very rapidly and proliferated at a rate that resulted in a maximum population comparable to the second maximum of the "0.01 mg cells" and "0.01 N cells" at the same time. These cells appeared normal and healthy.

Earle's strain L cells:

Replicate cultures of the second series of L cells were set up after the cells had been exposed for 110 days. The initial inoculum varied among the sets. For the controls, the inoculum was approximately 360,000 cells per ml; for the "0.01 mg cells", nearly 530,000 per ml; for the "0.05 mg cells", nearly 450,000 per ml. As in the second series of Liver cells part of the "0.01 mg cultures" and "0.05 mg cultures" were returned to regular (untreated) medium at the first medium change. The results obtained in this experiment were almost identical to those obtained in the first series. Proliferation rates for all cells were almost the same for the first two weeks. Differences appearing after that were probably of no great significance. All cells appeared to be in good condition. There was no evidence of inhibitory or stimulatory effects of the acetaldehyde.

HeLa cells:

Replicate cultures of HeLa cells were made after the cells had been exposed for 125 days. The inoculum for all the cultures was approximately 275,000 cells per ml. Part of the "0.01 mg cultures" and "0.05 mg cultures" were returned to regular medium at the first medium change.

Proliferation rates for all the cells did not vary greatly during the first seven days. At this time, however, the proliferation rates for the "0.01 mg cells"and "0.05 mg cells" slowed appreciably. This lag in proliferation of the "0.01 mg cells" and the "0.05 N cells" extended over a period of approximately a week; for the "0.05 mg cells" it extended throughout the remainder of the experiment.

1003541656

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Depletion of the cultures of the "0.05 N cells" make it difficult to predict the trend for these cells. There appears the possibility that the "0.01 mg cells" would reach a final population approximating that of the controls. The rates of proliferation for the controls and "0.01 N cells" closely approximated each other and exceeded the rates for the other cells. In the case of the "0.05 mg cells", the acetaldehyde has a definite influence; the rate of proliferation being less and the final population being about one-half that of the controls.

In appearance, the cells at first appeared to be healthy and typically young. As the cultures aged, the cells assumed the typical appearance found in older, crowded cultures. This was true except for the aging "0.05 mg cells" which did not cover the floor of the flask densely and hence were not very crowded; in fact, they appeared rather slender and many had long extensions.

10035416S7

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

194

Application For Research Grant

Date: January 28, 1958

1. Name of Investigator: Donald M. Pace, M.D.

2. Title: Professor & Chairman, Department of Physiology and Director, Institute for Cellular Research

3. Institution & Address: University of Nebraska, Lincoln, Nebraska

4. Project or Subject: To study the effects of certain constituents of tobacco smoke on tissue cells cultivated in vitro.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

We wish to study the effects of certain constituents of tobacco smoke on several types of tissue cells over short and long periods of time.

We are currently using the following well-established clones of cells: Earle's strain L fibroblasts, human skin cells, liver cells, and the Hela strain. It is also our desire to obtain a strain of lung epithelial cells (human) with the possibility of obtaining a clone.

Studies on the effects of air pollutants on these cell clones have been in progress during the past 1½ years.

Our specific aims, if the present request is granted, are:

(1) To study the effects of certain single smoke constituents on the various strains of cells as well as the effects of various concentrations of oxygen. The effects of mixtures of these smoke constituents with various concentrations of oxygen will also be observed.

(2) To culture lung tissue (according to the "organ culture" techniques) in order to study the effects of the smoke constituents on organized tissue.

The various cell types will be cultivated in as nearly a chemically-defined medium as possible. In preliminary investigations Earle's medium 109 plus 10% horse serum has been used.

The first part of these investigations will consist of exposing replicate cultures of the respective cell clones to various concentrations of a single constituent of smoke. Both periodic and continuous exposures will be made.

1003541658

Special flasks and delivery chambers have been devised so that proper mixtures of these gases can be made and applied to each individual culture.

The second part of the study will be concerned with investigations on the respiratory metabolism of the cells under these different conditions. Perhaps some clues as to the mechanism affected by these substances might be obtained.

The third part of the study will be made in parallel with the first two and will consist of observations on morphological changes that may take place within the cultures or within the cells themselves. This will be accomplished by time-lapse photomicrography so that a permanent record of the effects may be obtained.

1003541959

Source: https://www.industrydocuments.ucsf.edu/docs/rfvn0000

6. Budget Plan:

Salaries	$7000.00	($5500
Expendable Supplies	750.00	($1500
Permanent Equipment		
Overhead		
Other (Travel)	250.00	
Total	8000.00	

7. Anticipated Duration of Work: 1 year

8. Facilities and Staff Available: A laboratory consisting of a suite of 8 rooms fully equipped for short and long term tissue culture investigation.

There are several persons on the staff at present supported by other grants and University funds. These include a Chief Investigator, Co-investigator, Research Associate, Secretary-technician and laboratory helper.

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

Previous and current sources of funds have been awarded by 1) U.S.P.H.S. (grants through National Cancer Institute). 2) U.S.P.H.S, Air Pollution Medical Program (contracts). 3) American Cancer Society. 4) Tobacco Industry Research Committee (Fellowship). 5) Cooper Foundation.

We are at present working on a project designed to give information concerning the effects of air pollutants on Tissue cells cultivated in vitro. This work has actually been in progress for the past 1½ years and at this time we are just about ready to carry out studies on the effects of hydrocarbons and ozonides of same.

1003541660

Signature /s/ Donald M. Pace
Director of Project

/s/ Carl A. Donaldson
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/fmn0048

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

#194R1

(Originally activated Nov. 1, 1958)

Application For Research Grant

Date: August 5, 1959

1. Name of Investigator: Donald M. Pace, Ph.D.

2. Title: Professor of Physiology

3. Institution & Address: University of Nebraska
Lincoln, Nebraska

4. Project or Subject: A continuation of investigations into the effects of certain constituents of tobacco smoke on tissue cells cultivated in vitro, especially human lung and skin cells.

5. Detailed Plan of Procedure: The same procedures (tissue culture procedures) as given in the previous applications will be followed. However, more attention will be given to long range effects of selected substances in concentrations that appear to have no effect over short periods of time, as well as these effects on human cells, which have not yet been investigated from this point of view.

6. Budget Plan:

Salaries	$8,000.00	(6,000.) (2,000.)
Expendable Supplies	1,250.00	
Permanent Equipment	--------	
Overhead	--------	
Other (travel)	750.00	
Total	$10,000.00	

7. Anticipated Duration of Work: 1 year (or more)

8. Facilities and Staff Available: The Institute for Cellular Research consists of a suite of 8 rooms well equipped for short and long term tissue culture investigations. Although the above request is made for the support of one investigator and a part-time assistant, actually all the staff members of the Institute enter into some phase of the work.

9. Additional Requirements: We are using a synthetic nutrient medium (plus 10% horse serum) in which to cultivate our cells. The expendable supplies consist of rather expensive chemicals for the synthetic media and special glassware such as culture flasks, etc.

1003541661

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

10. Additional Information (Including relation of work to other projects and other sources of supply):

As stated in a previous request, we are also working on a project designed to give information concerning the effects of air pollutants on tissue cells cultivated in vitro. Ozone, hydrocarbons, SO_2, and NO_2 have been under observation. We have devised means for applying substances in the gaseous phase which can be used in our work on tobacco smoke.

/s/ Donald M. Pace
Director of Project

/s/ Perry W. Branch
Director-Secretary, University of Nebraska Foundation

1003541662

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Bioassay, carcinogenesis and tissue culture

#194R2

Comm:
Drs. Little, Chm.
Lynch
Jacobson
Kotin

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

Activated: 11/1/58
Renewed: 11/1/59

Application for Research Grant

Date: August 9, 1960

1. Name of Investigator: Donald M. Pace, Ph.D.

2. Title: Professor of Physiology

3. Institution & Address: University of Nebraska
Lincoln, Nebraska

4. Project or Subject: A continuation of investigations into the effects of certain constituents of tobacco smoke on tissue cells cultivated in vitro, especially human lung and skin cells.

5. Detailed Plan of Procedure: The same procedure as given in the previous application will be followed. Quantitative replicate cell culture techniques will be employed to study the effect of various constituents of cigarette smoke upon several cell strains. Emphasis will be placed upon long term exposure of the cells to sub-lethal concentrations of the various constituents.

A strain of human lung epithelial cells has been adapted to our replicate culture technique. Efforts will be made to clone this cell line. This should be of considerable usefulness in tobacco research.

Attempts will also be made to adapt a special technique devised for air pollution research in our laboratory so that replicate cell cultures may be directly exposed to whole tobacco smoke.

6. Budget Plan:

Salaries	$ 8,000
Expendable Supplies	1,400
Permanent Equipment	
Overhead (8%)	800
Other	600
Total	$10,800

7. Anticipated Duration of Work: One year (or more).

1003541663

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

8. Facilities and Staff Available: The Institute for Cellular Research consists of a suite of 8 rooms well equipped for both long term and short term tissue culture procedures. In addition to a director of research, there are two research associates, several research assistants and technicians. The staff is at present supported by research grants and University funds. This request would support one investigator and a part-time assistant.

9. Additional Requirements: No major additional requirements are anticipated. It should be pointed out that our cell culture methods employ a chemically defined medium supplemented with small amounts of horse serum. Such media contains rather expensive chemicals. The $1400.00 listed for expendable supplies would be used for such purchases. Special glassware, if needed, would also be purchased from this category.

10. Additional Information (Including relation of work to other projects and other sources of supply):

There are other research projects in our laboratory which should complement and supplement the work in the tobacco research area. Air pollution research projects have included studies employing NO_2, SO_2, ozone, hydrocarbon vapors, and combinations of these single constituents. Several publications in this area are in preparation and should appear in the literature soon.

An earlier investigation for T.I.R.C. using acetaldehyde has been completed and has been reported in CANCER RESEARCH, V. 20, No. 6, 1960 under the title "Studies on the Effects of Acetaldehyde on Tissue Cells Cultivated in Vitro" by D. M. Pace and Alice Elliott.

Investigations have been undertaken but as yet are incomplete on such tobacco constituents as: Formaldehyde, acetone, toluene, and methanol. The sodium salts of steam volatile fatty acids, such as formate, acetate, butyrate, valerate and caprate are also being tested. These experiments have not been completed but are mentioned to indicate the numbers of smoke constituents currently being studied.

/s/ Donald M. Pace
Director of Project

/s/ John K. Selleck?
President
University of Nebraska Foundation

1003541664

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N.Y.

113

Application For Research Grant

Date: September 26, 1955

1. Name of Investigator: Dr. John L. Patterson, Jr.
Dr. Wyndham B. Blanton, Jr.

2. Title: Associate Professor of Medicine, MCV
Assistant to the Dean MCV } Co-investigators

3. Institution & Address: Medical College of Virginia,
1200 East Broad Street,
Richmond 19, Virginia.

4. Project or Subject: Detection and Prognostic Significance of Early Obstructive Pulmonary Disease.

1003541665

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Introductory Remarks:

The most common and incapacitating form of chronic pulmonary disease of the later years is chronic pulmonary emphysema. The view usually advanced regarding its pathogenesis centers about the effect of long standing and progressive airway obstruction, bronchiolar obstruction being considered the most important because of the high airflow resistance in small tubes. Most of the reported studies on the pathological ~~xxx~~ physiology of this disease have been concerned with the stages in which definite clinical symptoms and signs are recognizable, i.e. usually moderate to far advanced disease.

In light of present knowledge of the pathology of advanced pulmonary emphysema, it is reasonable to assume that the only effective way of combating the later disability is via prophylactic or very early therapeutic measures. To apply such methods will necessitate the identification of individuals with early obstructive disease. The proposed investigations were conceived under the hypothesis that with proper methods it should be possible to detect early, perhaps definitely pre-clinical, degrees of bronchiolar obstruction.

In order to test the validity of this hypothesis, it would appear desirable, at least in the initial studies, to make observations on a ~~xx~~ group of subjects whose bronchial and bronchiolar involvement might be improved in a high percentage of cases. Subject groups for consideration are moderate to heavy users of tobacco before and after cessation of smoking, patients with allergic bronchitis, patients with reversible chronic infectious bronchitis and individuals whose occupations subject them to the inhalation of irritant gases. The group of chronic smokers would seem to be the most favorable group for immediate study, since they offer

Source: http://www.industrydocuments.ucsf.edu/docs/nfnd0000

the most readily controlled means of alleviating the bronchitis by cessation of smoking.

Plan of Procedure:

The common methods of pulmonary function study in most cases do not appear applicable to the problem of detection of minimal bronchiolar obstruction. A possible exception to this is the well-known Maximum Breathing Capacity, although the significance of small changes in this measurement are difficult to evaluate and its usefulness in this type of study is doubtful. A reasonable approach would be to place the airway system under maximal stress by having the individual carry out maximal expiratory and inspiratory effort during which airway resistance would be studied. One measure of this resistance can be obtained by continuously recording the trans-pulmonary pressure gradient, i.e. the gradient from the surface of the lung to the mouth, together with simultaneous recording of instantaneous velocity of airflow during the expiratory effort. It is proposed to measure velocity of airflow by means of a screen type of flowmeter of sufficient diameter to permit linearity of response up to 600 liters per minute. Intrathoracic pressure, or an approximation to it, can be obtained from a small diameter esophageal balloon.(1) It is felt that under the conditions of this experiment, i.e. maximal expulsive effort, the admitted error due to esophageal tone (noted in quiet breathing) should not seriously affect the results in view of the total pressure being measured. Since resistance to airflow with laminar flow tends to be proportional to velocity of flow, whereas resistance in the case of turbulent flow is proportional to the square of the velocity, the method described above should be particularly sensitive in the detection of small decreases in airway caliber if these are associated with increased turbulence. The type of pathologic changes which we believe occur in the tracheobronchial tree in the conditions to be studied leads us to anticipate increase in turbulent flow.

The equipment will be designed for simultaneous use with more simple apparatus for measurement of respiratory function so that the possible utility of the latter in detection of early obstructive disease can be evaluated. There has been developed in this laboratory a simple type of recording spirometer with minimal friction and inertia properties which, it is hoped, will prove useful for this purpose.

A somewhat different approach to the detection of early bronchiolar obstruction is the measurement of the effects of such obstruction on the equality of inequality of distribution of inspired air throughout the lung. Techniques are available at present for measuring defective intra-pulmonary gas mixing by the continuous analysis of helium concentration in expired gas after equilibration of the subject on a helium-oxygen mixture. (2) or of expired nitrogen concentration after a single breath of 100% oxygen.(3) The sensitivity of both the nitrogen and helium methods leads us to anticipate detectable changes in these measures of pulmonary air distribution in the presence of early sub-clinical airway obstruction.

It is planned to correlate the findings determined by resistance, air distribution, and simple respirometer methods.

The plan of procedure is as follows: Control studies on given subjects will be done repetitively for consistency of findings after detailed clinical examination. When these are completed, the measure designed to diminish or relieve the bronchiolar obstruction, for example, cessation of smoking, will be instituted followed by serial studies at appropriate intervals until maximal change has been achieved. The attempt will then be made in some subjects to restore the original status, which would involve, in the example cited, reinstitution of smoking and again a series of experimental studies. The number of subjects required for the study will depend upon the consistency of the findings and cannot be estimated with certainty, but a working figure of twenty subjects appears reasonable.

No chest x-ray or fluoroscopic examination

Probl 1st ex. after rest until discharge

1003541666

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

1. Mead, J., McIlroy, M.D., Selverstone, N. J., and Kriete, B. C., Measurement of Intraesophageal Pressure, Journal of Applied Physiology, 7:491,1955.

2. Blair, E. and Hickam, J.B., Quantitative Study of Intrapulmonary Gas Mixing in Emphysema, American Journal of Medicine, 18:519, 1955.

3. Comroe, J. H. and Fowler, W. S., Lung Function Studies: VI. Detection of Uneven Alveolar Ventilation during a Single Breath of Oxygen, American Journal of Medicine, 10:408, 1951.

1003541667

Source: https://www.industrydocuments.ucsf.edu/docs/fvrh0000

6. Budget Plan:

Salaries	$1,380.00
Expendable Supplies	618.04
Permanent Equipment	2,815.00*
Overhead	886.96
Other	1,100.00**
Total	$6,800.00

7. Anticipated Duration of Work: One Year.

8. Facilities and Staff Available:

The facilities of the Pulmonary Function Laboratory of the Medical College of Virginia are available to this project. The plan calls for assighment of approximately one-half the time of a qualified technician to work solely with this project, under the supervisioh of the investigators named above.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

This work will be carried out in the facilities of the Medical College of Virginia Pulmonary Laboratory which operates in close conjunction with the Cardiovascular Research Laboratories. At the present time a number of other investigative studies in the field of cardio-respiratory physiology are being carried out by these groups and the advice of and, to some extent, the time of the personnel involved will be available as necessary.

*Permanent Equipment:

Flowmeter:	
Differential Strain Gauge	$350.00
Differential Strain Gauge Amplifier	350.00
Screen and Housing	100.00
Nitrogen Meter (The Waters Corp.)	1,675.00
Helium Kathorometer (Gow-Mac Instrument Corp.) With Housing	70.00
Leeds-Northrup Potentiometer double acale range 0-16 and 16-64 m.volts	270.00

**Other:

Paid Subjects	$800.00
Cost of X-ray	300.00

s/ W. B. Blanton, Jr.

Signature s/ John L. Patterson, Jr.
Director of Project

s/ W. F. Tompkins
Business Officer of the Institution

W. F. Tompkins

10035468

Source: https://www.industrydocuments.ucsf.edu/docs/wvnd0068

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Application For Research Grant

Date: May 13, 1955

1. Name of Investigator: John M. Patterson, Assistant Professor) Co-directors and
Walter T. Smith, Associate Professor) principal investigators

2. Title:

3. Institution & Address: Kentucky Research Foundation
for:
Department of Chemistry
University of Kentucky
Lexington, Kentucky

4. Project or Subject: Fundamental Approaches to the Evaluation of Tobacco in Regard to Quality and Physiological Properties.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed)

I. Investigation of the Non-alkaloid Fractions of Tobacco.

In addition to the alkaloids, the following types of compounds have been found in tobacco: protein, reducing sugars, reducing substances other than sugars, sucrose, dextrin, pectic substances, pentosans, cellulose, lignin, polyphenols, tannins, resins, and waxes. Certain classes of these compounds will be investigated with respect to their carcinogenic activity. In such a study the resins might be a particularly fruitful field since compounds of somewhat related structure are known to be carcinogenic. The role of terpenes in tobacco will also be investigated. It is anticipated that these studies will be carried out on genetically controlled strains of tobacco which will be made available to us by the Kentucky Agricultural Experiment Station.

II. An Investigation of the Alkaloid Fractions:

This would be divided into two parts. (1) A study of the ratio of the different alkaloids in various strains, and a correlation of the ratio with the quality and physiological properties of the tobacco. (2) A search for unreported alkaloids in new strains of tobacco. The formation of new alkaloids may have a pronounced effect on the quality of the tobacco. It is anticipated that these studies will be carried out on genetically controlled strains of tobacco which will be made available to us by the Kentucky Agricultural Experiment Station.

2085416

III. Pyrolysis of Known Pure Compounds Found in Tobacco.

This project is concerned with the pyrolytic behavior of pure compounds, particularly at the temperature of a burning cigarette. The pyrolysis would be studied in an inert atmosphere and also in an atmosphere containing varying amounts of oxygen and moisture. The products of pyrolysis would be examined for the presence of physiologically active substances, particularly those compounds which influence the cardiovascular system and uncontrolled cell growth.

The use of varying amounts of oxygen and moisture is of significance because the nature of the pyrolysis products might be fundamentally changed when the amount of these substances is varied. Thus this might point the way to the elimination of possible harmful substances in tobacco smoke simply by a slight change in the conditions under which the tobacco is smoked.

This approach is unique in that pure compounds or known mixtures of pure compounds are subjected to conditions similar to that in burnigg tobacco. This represents a fundamental approach to the problem and permits a study of the interaction of the various components of burning tobacco.

This project will also be extended to include the pyrolysis of simple, model compounds of the naturally occurring substances.

1003516670

Source: https://www.industrydocumentslibrary.ucsf.edu/tobacco

6. Budget Plan:

Salaries	$11,516.00
Expendable Supplies	1,500.00
Permanent Equipment	12,255.00
Overhead	5,412.52
Other	200.00
Total	$30,883.52

7. Anticipated Duration of Work: The above budget is for the first year. The projects outlined above should be approaching completion in about three years. For estimated budget after first year, see Exhibit A.

8. Facilities and Staff Available: The facilities of the Department of Chemistry, including ordinary chemicals, equipment, and special instruments, are available for this work.

The research assistants to be employed for these projects will be qualified graduate students working toward advanced degrees.

9. Additional Requirements:

1. Baird Infrared Spectrophotometer
2. Pyrometer Controller
(see detailed budget page.)

10. Additional Information (Including relation of work to other projects and other sources of supply):

A project involving pyrolytic studies, and directed by one of us, is durrently in progress. The experience derived will be valuable in pursuance of the proposals outlined above.

1003541621

Signature /s/ John M. Patterson
/s/ Walter T. Smith, Jr.
Director of Project

/s/ Merl Baker
Business Officer of the Institution

Director, Kentucky Research Foundation

Source: https://www.industrydocuments.ucsf.edu/docs/ttvn0000

Exhibit A

Detailed Budget for One Year

Personnel		
Professional:		
John M. Patterson, W. T. Smith, Jr. — Directors and Principal Investigators	$ 900.00 900.00	
The directors will be released from regular duties during the regular school year to an extent sufficient to allow time for the proposed research.		
Research Assistants		
Project I (1 assistant)	1,800.00	
Project II (2 assistants)	3,600.00	
Project III (3 assistants)	5,400.00	
Social Security for above salaries	216.00	
Non-professional:		
Student labor		
500 hours at $1.00 per hour	500.00	
Sub-total		$11,516.00
Instruments and Permanent Equipment		
Baird Infrared Spectrophotometer	12,000.00*	
All of these projects involve elucidation of structures and identification of organic compounds. The spectrophotometer would be a highly valuable, if not necessary, tool for the accomplishment of this purpose.		
(* This represents the f.o.b. price. The Department of Chemistry will absorb transportation costs and other costs incidental to putting the apparatus into operation.)		
Pyrometer Controller	255.00	
Sub-total		$12,255.00
Materials and Expendable Supplies		
Special Chemicals and Elemental Analyses		1,500.00
Travel and Communication		200.00
Overhead		5,412.52
Total		$30,883.52

only a few tools in U.S. of almost equivalent

Note: The budget for succeeding years would not include the item for Instruments and Permanent Equipment and would amount to $18,628.52.

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Source: http://www.industrydocuments.ucsf.edu/tobacco/

195

From the desk of:

ROBERT N. DU PUIS

To:

5. I. How investigate for carcogenicity?
" Know structure-resins?
What role terpenes in tobacco?
What genetically controlled strains tobacco? Should be commercially acceptable varieties.

II. alkaloid study very difficult + complex. Jeffries has done most of this.
How determine quality? TLC?
" " physiol. props?
How relate new alkaloids to quality? Do this TLC?

III. How pyrolyze?
How separate + identify products?
V. diff. + complex. Do they know gas chromat?
IR will be unsophisticated.
How test effects on cardio-vascular system + cell growth.

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Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]0000

195

From the desk of:

ROBERT N. DU PUIS

To:

ultimately must pyrolyze tobacco
itself & identify products
Complex interactions
What are "simple model cpds.
Do naturally occurring
substances?

General:

This is no project for the
uninitiated especially
graduate students
Much too naive.
Shot gun approach - too few
details & technique.
Glib reference to physical tests
I & spec. quite inadequate
as only major tool.

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Source: https://www.industrydocuments.ucsf.edu/docs/tkvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y. #177

Application For Research Grant

Date: August 8, 1957

1. Name of Investigator: Arthur R. Payzant, M.D.

2. Title: Chief of the Radiology Department

3. Institution Touro Infirmary
& Address: Prytania at Delachaise Sts., New Orleans 15, La.

4. Project or Subject: Determination of roentgenographic changes in the plain PPA of the chest, if any, of vascular shadows as related to smoking of cigarettes, correlated to length of time of smoking and type of smoking habits.

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5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

The basic research will be conducted by Dr. Josue Pagan-Carlo, Radiology Resident, under the supervision of Dr. Payzant, Chief Radiologist; Dr. T. J. Haley, and Dr. George A. Gentner, Associate Radiologists. Dr. Pagan-Carlo will be allowed a subsistence stipend of $300.00 a month from the Grant-in-Aid fund.

The program will consist of an evaluation of all routine chest films, PPA views, and when necessary tomographic cuts at the level of the tracheal carina, of the thickness of the vascular markings at the mid pulmonary and at the peripheral pulmonary zones of smokers and non-smokers. Chest films taken in patients with known or suspected cardiopulmonary pathology, other than carcinomas of the lungs,, will not be evaluated. The findings will be tabulated by type of smoking, length of time of smoking and amount of smoking, age, and sex. The average thickness of the markings in known normal chests will be compared with those of smokers classified as light, moderate, heavy, depending on the number of cigarettes smoked daily. Any statistical significant deviation will be determined by currently used statistical methods.

It is estimated that over 2,000 films will be reviewed in the course of one year, involving both the normal non-smoker chest and the smoker's chests. Micrometric measurements will be used to assure an accurate measurement as possible. Special tomographic examinations and additional views necessary on any case will be paid out of the Grant-in-Aid fund to the hospital.

Source: https://www.industrydocuments.ucsf.edu/docs/tfkn0000

5. Detailed Plan of Procedure (Cont.)

The final evaluation as to the thickness and patterns of the vascular markings will be on the basis of cooperative examination of the films by the Chief Radiologist, the associate radiologists, and the radiology resident. The technicians taking the films will be instructed to obtain the necessary smoking history from the patient as well as the Chief receptionist, as specifically instructed by Dr. Pagan-Carlo; for this additional work they will receive a nominal compensation from the Grant-in-Aid fund.

The necessary photographic work and clerical work for the publication of the results of this research program also will be paid out of Grant-in-Aid funds. If desired necessary, a special clinical photography camera will be purchased and will become part of the permanent equipment of the Radiology Department, this camera will be used to copy selected films of the chest.

The project will last an estimated 12 months.

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Source: https://www.industrydocuments.ucsf.edu/docs/MNg0000

6. Budget Plan:

Salaries	$ 4,100.00
Expendable Supplies	100.00
Permanent Equipment	500.00
Overhead	
Other	300.00
Total	$ 5,000.00

7. Anticipated Duration of Work: 12 months

8. Facilities and Staff Available:

The facilities of the X-Ray Department of Touro Infirmary will be used. Over 100 diagnostic cases is the daily load of the department (15% of which are chest x-rays). There are two fluoroscopic machines, 200 MA; four diagnostic machines including two 500 MA; one special tomographic and laminagraphic machine; other special equipment of the department used in rapid serial x-ray exposures for angiocardiography and arteriography are available but will not be used. The department has 5 technicians, and 5 students of x-ray technic, two dark-room technicians, ~~and~~ from Incidentally the darkroom has timed and thermostatically controlled developer for uniform film development. (See reverse side for continuation.)

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Touro Infirmary has over 500 beds (this includes 48 bassinets), with a very active out-patient clinic and teaching ward service. The hospital is approved by the American Medical Association for the graduate training of residents in many specialty fields, it is approved for intern training, accredited by the Joint Commission on Hospital Accreditation. Touro Infirmary is affiliated with Tulane University School of Medicine for the training of Medical Students in clinical medicine.

Signature _____
Director of Project Arthur P. Fayzant, M.D.
CHIEF RADIOLOGIST

_____
Business Officer of the Institution John C. Mackenzie, M.D.

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Source: https://www.industrydocuments.ucsf.edu/docs/MYX00000

8. Facilities and Staff Available (Cont.)

There are three full time radiologists, all certified in Radiology by the American Board of Radiology. The Department is approved for the training of residents in Radiology by the American Medical Association and by the American Medical Board of Directors of Radiology. It is also approved for fellowship training by the United Public Health Service, National Institute of Cancer and by the American Cancer Society.

The Department also has a school of X-Ray Technicians approved by the American Registry of X-Ray Technicians and by the American Medical Association.

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Source: https://www.industrydocuments.ucsf.edu/docs/hyrn0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

111

Application For Research Grant

(110)

Date: September 19, 1955

1. Name of Investigator: Samuel J. Pearlman, M.D. - Ira J. Tresley, M.D. - Otto Saphir, M.D.
2. Title: Samuel J. Pearlman-Attending Surgeon, Ear Nose and Throat Department, Michael Reese Hospital-Attending Surgeon, Chairman of Dept. of Ear Nose and Throat, Cook County Hospital-Associate Professor, Ear Nose and Throat Dept., Northwestern University School of Medicine.
Otto Saphir-Prof. Dept. of Pathology, U.of Illinois School of Medicine-Director
3. Institution & Address: Department of Pathology, Michael Reese Hospital.
Ira J. ~~Yaxx~~R Tresley-Associate Surgeon, Ear Nose and Throat Dept. Cook County Hospital-Associate in Ear Nose and Throat Dept., Northwestern U. School of Medicine.
3.---Michael Reese Hospital, 29th & Ellis Avenue, Chicago, Illinois
4. Project or Subject:

To determine the normal by study of exfoliated cells in the area of the Nose, Throat and Bronchi, in patients of all ages, and in both ~~as~~ sexes.
To transmit these findings to the Ear Nose and Throat Specialist by way of his special journals.
To make a limited study of older persons, primarily non-smokers and heavy smokers.

This ~~week~~ last would consist of a study of exfoliated material from the Nose, Lips, Pharynx, Tongue, Larynx and Bronchial Tract.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed)

Our methods would be those already in use, utilizing the staining methods of Papanicolaou. In addition, phase microscopy would be at our disposal. A combination of Papanicolaou stained smears and phase microscopy we believe offers some interesting possibilities. Our material would come from our private practices and the large Clinical material offered by the Cook County Hospital. As Clinicians we do not claim to be expert in the above examinations.

Dr. Saphir is an accepted authority, however, and maintains a laboratory at the Michael Reese Hospital with competent technicians.

We do not intend this study to be a search for early or undiscovered carcinomas. Much has already been done along these lines, especially in the broncho pulmonary area.

Very few clinicians can hope to become excellent Cytologists, but they should be informed of the normal and understood basic principles.

Contrary to the case in Gynecology the field of Nose and Throat requires much educative work.

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Source: https://www.industrydocuments.ucsf.edu/docs/xbmc0000

6. Budget Plan:

Salaries	including photography	$4,000.00
Expendable Supplies		2,500.00
Permanent Equipment		none
Overhead		none
Other		1,000.00
Total		$7,500.00

7. Anticipated Duration of Work:

One year

8. Facilities and Staff Available:

The Pathological laboratory of the Michael Reese Hospital, including the supervision of the Director of the Pathology Department, and his staff of medical men and technicians.

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9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

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s/ Samuel J. Pearlman, M.D.

Signature

Director of Project

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/fbm0000

#170R2

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

(Cf. #170 - Activated: 2/1/58
Renewed: 2/1/'59)

Renewal
Application for Research Grant

Date: December 8, 1959

1. Name of Investigator: Edward W. Pelikan, M.D.

2. Title: Associate Professor of Pharmacology

3. Institution & Address: Department of Pharmacology and Experimental Therapeutics
Boston University School of Medicine
80 East Concord Street
Boston 18, Massachusetts

4. Project or Subject: Pharmacological analysis of the mechanism by which nicotine impairs transmission through autonomic ganglia.

5. Detailed Plan of Procedure: In previous reports of the present project, we described the evidence which lead us to believe that nicotine prevents transmission of nerve impulses through autonomic ganglia by a mechanism hitherto never ascribed to nicotine. In brief, we believe that nicotine can modify cardiovascular and other autonomic effector activity by preventing release of acetylcholine from nerve endings in autonomic ganglia, and thereby prevent nerve impulse transmission. We have not attempted to investigate the mechanism by which nicotine causes stimulation of autonomic ganglia.

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The data upon which this hypothesis is based were obtained by recording the mechanical responses of the cat nictitating membrane to high and low frequencies of preganglionic nerve stimulation. These data were compared with mathematical formulations of effects to be expected were a hypothetical drug to impair ganglionic transmission by impairing acetylcholine release or, alternatively, by preventing access of acetylcholine to postsynaptic cell receptors. The excellent fit of the data obtained with nicotine, tetraethylammonium ions, morphine, and hexamethonium ions to the theoretical formulations led to our establishing the hypothesis summarized above.

During the past year the over-all object of our work has been to obtain direct experimental evidence that impaired ganglionic transmission produced by nicotine, tetraethylammonium or morphine was accompanied by a decrease in the rate of release of acetylcholine from the ganglion, and that hexamethonium blocked ganglionic transmission without affecting acetylcholine release. The work was performed in two parts:

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

(1) A method was developed, using the isolated rat ileal strip, which permitted the bioassay of quantities of acetylcholine as low as one one-thousandth of a microgram. The assays could be performed without interference by the presence of nicotine, tetraethylammonium, or hexamethonium in quantities one thousand to one hundred thousand times as large. Using this technique it was possible to demonstrate that nicotine in concentrations of 10 mcg/ml completely suppressed the release of acetylcholine from isolated guinea pig ileal segments; no such suppression of acetylcholine release was obtained with concentrations of tetraethylammonium or hexamethonium as large as 1 mg/ml. A summary of this work was submitted to the Scientific Advisory Board on May 7, 1959.

(2) The superior cervical ganglion of the anesthetized cat was isolated and perfused with Ringer's solution using, essentially, the method of Kibjakov. Using this technique, the ganglion and its nerve supply, and the nerve supply to the nictitating membrane remain functional for reasonably long periods of time. The effluent from the ganglion was collected before, during, and after various procedures, including the injection of nicotine, and the acetylcholine content of each sample was assayed using the bioassay technique described above. A brief summary of the experimental results is appended to this application; a detailed summary will be submitted upon the expiration of the current research grant.

We propose to continue our research by extending our investigations of the effects of nicotine and other ganglionic "blocking" agents on the output of acetylcholine from the isolated, perfused superior cervical ganglion of the cat. Specifically, at least four kinds of experiments need to be performed:

1. Additional experiments must be performed to confirm the observed effects of nicotine in decreasing the output of acetylcholine from the ganglion. At the present time, satisfactory experiments with nicotine have been performed in three cats; for only two experiments could the results of the bioassay be interpreted unequivocally. For both experiments, the data indicate that acetylcholine output from the ganglion was decreased abruptly and remarkably at the time ganglionic transmission was prevented by the injected nicotine.

2. Experiments must be performed with agents such as morphine and tetraethylammonium, which we anticipate will prevent ganglionic transmission and decrease acetylcholine output as nicotine seems to do. Experiments must be performed using hexamethonium to produce ganglionic blockade; we anticipate such a blockade to be unaccompanied by significant changes in acetylcholine release. In one experiment performed so far with hexamethonium, it was found that the acetylcholine content of the effluent from the ganglion was too low, even before drug administration, to permit reliable assays.

   It is obviously crucial to our hypothesis concerning the mechanism of action of nicotine in preventing ganglionic transmission that we demonstrate that agents which affect the response of the nictitating membrane to high and low frequency stimulation, as nicotine does, also have effects on acetylcholine output like those of nicotine. Similarly, a counter proof is required; hexamethonium and nicotine affect the response of the membrane differently, and we must demonstrate different effects of the two on acetylcholine output.

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Source: https://www.industrydocuments.ucsf.edu/docs/fvm0000

3. Experiments must be performed to determine the significance of changes in vascularity in the perfused tissues in altering the quantity of acetylcholine in the effluent. In contrast to our expectation based on a study of the literature, we have found it impossible, thus far, to isolate the afferent and efferent vascular supply of the ganglion from the systemic circulation, or from the circulation to other tissues which may act as potential sources at least of acetylcholine. Furthermore, we have observed that injection of nicotine to the ganglion causes changes in resistance to perfusion and changes in the fraction of infused fluid recovered as effluent; these results may indicate that acetylcholine detected in "control" samples of effluent may be derived in whole or in part from areas of the cat quite different from those which supply the acetylcholine which is detected in effluent collected during the course of the nicotine effect.

The effect of experimental manipulations in changing the areas of perfusion and drainage in the Kibjakov preparation and the consequent effects of such changes on the apparent output of "transmitter" from the "ganglion" seems not to have been discussed in previous publications. This is most remarkable since the theory of acetylcholine as the neurohumoral transmitter in ganglia rests in part on the inference that changes in acetylcholine output from the "ganglion" are related only to the functional state of pre-ganglionic nerve terminals in the ganglion alone.

We hope to investigate the relationship between the vascularity of the perfused area and acetylcholine output in experiments in which:

a. We study the effects of surgical extirpation of the ganglion or pre- and post-ganglionic nerve section on acetylcholine output and resistance to perfusion.

b. Vasoconstriction in the perfused area is produced by a substance such as vasopressin which has no known effects on acetylcholine release.

c. An attempt is made to influence acetylcholine output with agents such as morphine which may well be given without producing significant effects on the vasculature. Any effects of morphine on acetylcholine output may reasonably be expected to outlast effects on vessels such as might be produced by histamine release.

4. Experiments should be performed to identify the spasmogen assayed in our rat ileal preparation with acetylcholine. Like the activity of acetylcholine, the activity of the spasmogen we assay as acetylcholine is destroyed by boiling of the effluent or prevented by atropinization of the ileum. However, in our 4-point-assay design, dose-effect curves for the spasmogen have slopes consistently higher than the slopes of the curves for the standard, acetylcholine. According to the logic of bioassay, this indicates that the spasmogenic activity of the samples does not reside in a molecular species identical with acetylcholine. Previous investigators have routinely used 3-point-assays of the effluent to

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Source: https://www.industrydocuments.ucsf.edu/docs/fbm0000

detect "acetylcholine." Such a technique permits the use of small effluent volumes and permits a larger proportion of "successful" assays and experiments, but precludes identification of the standard with the unknown agent.

Resolution of the problem of the non-parallelism of the dose-effect curves of the standards and unknown agents may take two courses:

a. Alteration of the experimental design--by using other anesthetic agents, anti-coagulant drugs, or anti-cholinesterase drugs in the perfusing fluid--to determine whether the lack of parallelism is adventitious.

b. Concentration of the effluent and an attempt to identify acetylcholine in it by chemical or enzymatic means.

The methods presently available to us seem adequate to determining whether administration of nicotine, or other agents which impair ganglionic transmission, can alter both ganglionic transmission and "acetylcholine" output in the effluent. An adequate biological interpretation of these facts, an attempt to determine causal relations between them, requires further identification of the ganglion as the only significant source of the spasmogen assayed and more certain identification of the spasmogen with acetylcholine.

6. Budget Plan:

Salaries	$ 4,500. *
Expendable Supplies	2,000.
Permanent Equipment	2,000.
Overhead	1,365.
Other	600.
Total	$ 10,465.

* E. W. Pelikan $1,000.
Research Asst. 3,500.

7. Anticipated Duration of Work:

Two years; the budget plan above applies only to the first year of the research.

8. Facilities and Staff Available:

Facilities and staff of the Department of Pharmacology and Experimental Therapeutics of the Boston University School of Medicine. The staff members are competent and well equipped to aid the course of this study, especially insofar as special knowledge of biochemical and physical chemical methods might be required, and insofar as special techniques might be required to record and study cardiovascular phenomena. No salaries are requested for persons who might aid the work in this way.

9. Additional Requirements:

None.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

10. Additional Information (Including relation of work to other projects and other sources of supply):

This proposed research program is the direct outgrowth and continuation of research now being conducted by the applicant under a grant from the Tobacco Industry Research Committee. The work is also an extension of previous investigation by the applicant; these investigations have included studies of the pharmacology of the neuromuscular junction in several species, studies of the structure-activity relationships among neuromuscular blocking agents, and studies of the actions of nicotine and sympathomimetic amines on isolated intestinal strips, the frog rectus abdominis muscle, and on the superior cervical ganglion of the cat.

Application for support of this work has not been made to any other agencies.

The applicant will spend about three-fourths of his time on these investigations.

/s/ Edward W. Pelikan

/s/ K. M. Haetzfeld

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #170R1 Report #3

Edward W. Pelikan, M.D.
Boston University School of Medicine

December, 1959

A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms

A total of 22 experiments have been performed in barbitalized cats to determine the effect of agents which impair ganglionic transmission on the output of acetylcholine from the superior cervical ganglion. In all experiments, the superior cervical ganglion was perfused, essentially as first described by Kibjakov, using a warm, oxygenated, Ringer's solution containing neostigmine; the content of "transmitter substance" in the effluent from the ganglion was assayed as acetylcholine using the isolated rat ileal strip preparation we have described in an earlier report.

In five cats, death of the animal or injury to the ganglion precluded carrying out our anticipated experiments. Seven cats were used to develop the surgical and mechanical techniques used in the work. Four cats were used exclusively to study the vascular dynamics and behavior of the ganglion under the conditions of the experiment. In 5 experiments, in four of which ganglionic blocking drugs were administered, collection and bioassays were performed on effluent from the ganglion; in two of the three experiments with nicotine, behavior of both the donor preparation and of the assay preparation permitted adequate study of the effects of the drug on "transmitter" output.

Many of our results had not been anticipated, on the basis of our studies of the previous publications concerning this preparation; some of these unanticipated results are summarized in Figures 1 and 2, in which data from representative experiments are graphed; results of the two successful experiments with nicotine are graphed in Figures 3 and 4.

In contrast to our impression gained from the literature, we were unable to isolate the circulation to the ganglion from the systemic circulation. This fact is documented in Figure 1. Surgical isolation of the ganglion, complete at time "A" in the figure, never resulted in obtaining a blood-free effluent from the ganglion. In Figure 1 are plotted the hemoglobin concentrations of systemic venous blood and of effluent samples as a percent of initial systemic blood hemoglobin concentration (hemoglobin was determined spectrophotometrically in hemolyzed samples). Perfusion of the surgically "isolated" ganglion with Ringer's solution caused progressive dilution of systemic blood and a slight decrease in hemoglobin concentration of the effluent. Ligation of the major cervical vessels on the side contralateral to the infusion ("B" in Figure 1) permitted obtaining a lower hemoglobin concentration in the effluent than in the systemic blood. Only death of the cat ("C" in Figure 1) made it possible to obtain a hemoglobin-free effluent. Simultaneous with achievement of a blood-free effluent resistance to perfusion (mean pressure head/output of constant output pump) decreased. Although death of the cat prevented influx of blood into the perfusate, it did not prevent efflux of perfusate into the systemic vasculature, as indicated by the ratio (effluent (ml/min)/pump output (ml/min)) which, except during the period of asphyxia, was uniformly much less than one. At the end of the experiment shown in Figure 1, it was found that only about 17% of the total volume infused had been recovered in the effluent.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

All experiments with ganglionic blocking agents, designed to measure the effects of the agent on "transmitter" output, have been and will be performed on cats which are dead, systemically, in order to facilitate collection of effluent from the ganglion alone.

Although preganglionic nerve stimulation had no consistent effect on flow of perfusate through the ganglion (occasionally such stimulation was followed by the appearance of minute amounts of blood in the previously clear effluent) the administration of nicotine in doses known to prevent ganglionic transmission was followed regularly by changes in ganglionic perfusion. Such changes are represented in Figure 2. Retrograde intra-arterial injection of nicotine (100 mcg) caused a rise in resistance to perfusion and, in this case, a relative decrease in the amount of effluent (this is a reflection of an absolute decrease in the amount of effluent). These changes occurred regardless of ligation of the contralateral cervical vessels and regardless of the death of the cat. Communication between the perfusion system and circulatory system is demonstrated graphically in the remarkable change in systemic blood pressure produced by the first intra-arterial injection of nicotine.

Preganglionic nerve stimulation was not found to have consistent effects in increasing the output of substance spasmogenic to the rat ileum (Fig. 3 and 4). This is in contrast to data reported previously in the literature. However, like the activity of acetylcholine and like the activity of materials detected in ganglionic effluent by other investigators, spasmogenic activity in effluents obtained in our experiments is abolished by boiling the effluent and by atropinization of the assay preparation.

In Figures 3 and 4 are shown the results obtained in two experiments involving retrograde intra-arterial administration to the ganglion of doses of nicotine (100 mcg) which prevent ganglionic transmission. In both experiments, drug administration was followed by a decrease in output of spasmogen in the effluent; concomitant to this change was a decrease in response of the nictitating membrane to preganglionic stimulation. In both experiments, administration of nicotine was followed by an increased resistance to perfusion; in both experiments this increase in resistance was accompanied by a moderate increase in the fraction of perfusate recovered as effluent, although the absolute amount of effluent decreased in both experiments. It is as if injection of nicotine closed to perfusion vascular beds previously open and in communication with the total vascular system of the animal.

Comparison of Figures 3 and 4 indicates that perfusion is not necessarily accompanied by a progressive decline in spasmogen output. In both cases, however, injection of nicotine produced a sudden decrease in spasmogen output. It is conceivable, in light of the data, that the effect observed after nicotine was the result only of a failure of efflux from a nonganglionic site (but richer in spasmogen than efflux from the ganglion, per se) to gain access to the total effluent, and that the failure of the nictitating membrane to respond and the decrease in spasmogen output were related coincidentally rather than causally. In refutation of this point of view can be mentioned only that one experiment

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in which the usual experimental procedure was intentionally carried out on a cat in which the ganglion as well as the body was dead, indicated that no spasmogen was leeched from the tissues by perfusion, or that it was produced in quantities much less than 10^{-10} grams/minute.

With the reservations introduced by the effects of nicotine on the vasculature of the perfused tissues, our results are consistent with our original hypothesis that nicotine exerts its effects in impairing ganglionic transmission by decreasing the output of transmitter substance. Future experiments must include:

1. Additional studies to confirm the observed effects of nicotine on spasmogen output.

2. Experiments with other agents such as morphine or tetraethylammonium ion, which should affect transmitter output as nicotine is presumed to do, and experiments with hexamethonium ion which we anticipate will prevent ganglionic transmission without altering spasmogen output.

3. Experiments involving such procedures as surgical extirpation of the ganglion, or using drugs which decrease spasmogen output without affecting blood vessels in the area perfused, or alter blood vessel diameter without changing spasmogen output, in order to evaluate the significance of changes in vascular resistance in producing results such as we have obtained so far.

4. Experiments designed to determine the chemical nature of the spasmogen assayed in our preparation, or to identify it certainly with acetylcholine or other hypothesized chemical mediators of synaptic transmission.

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Source: http://www.industrydocuments.ucsf.edu/docs/thnn0000

FIGURE 1 PERFUSION OF CAT SUPERIOR CERVICAL GANGLION

AT "A", PERFUSION BEGUN; AT "B", LIGATION OF CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS; AT "C", CAT KILLED BY ASPHYXIATION. TOTAL VOLUME INFUSED: 403 ml.; TOTAL EFFLUENT FROM TRANSVERSE POSTERIOR FACIAL VEIN: 69 ml.

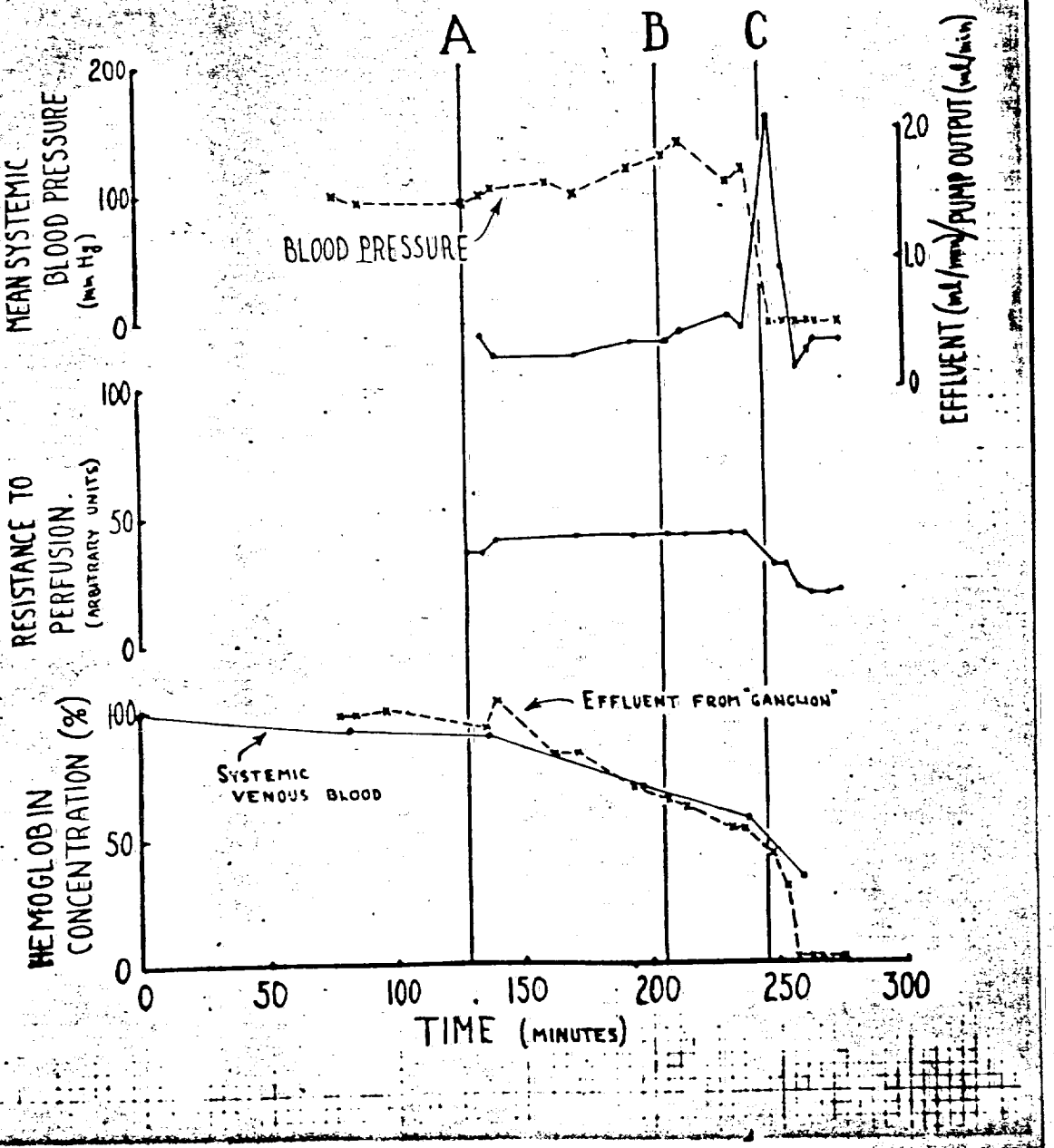

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FIGURE 2: PERFUSION OF CAT SUPERIOR CERVICAL GANGLION

AT "A", PERFUSION BEGUN; AT "B", CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS LIGATED AND CAT KILLED BY ASPHYXIATION.

AT "N", NICOTINE, 100 mcg, ADMINISTERED TO GANGLION BY INTRA-ARTERIAL INJECTION.

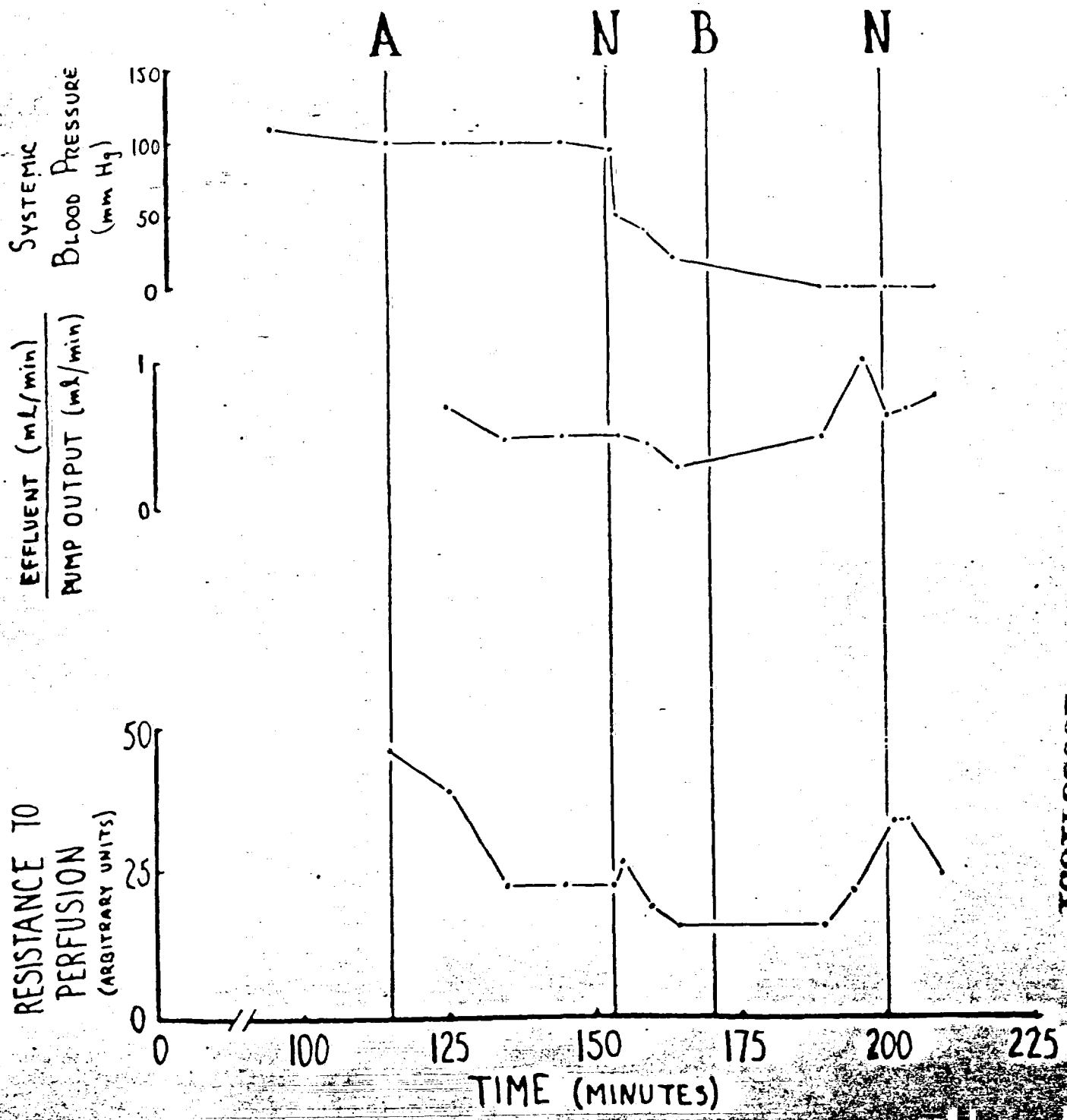

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FIGURE 3 EFFECT OF NICOTINE ON RATE OF RELEASE OF SPASMOGEN FROM PERFUSED SUPERIOR CERVICAL GANGLION OF CAT. CLOSED CIRCLES SHOW RESULTS OF ASSAYS IN DUPLICATE ON EFFLUENT OBTAINED DURING PRE-GANGLIONIC NERVE STIMULATION; OPEN CIRCLES INDICATE RESULTS OBTAINED WITHOUT STIMULATION. HEAVY HORIZONTAL BARS INDICATE GEOMETRIC MEANS OF REPLICATE ASSAYS AND DURATION OF COLLECTION PERIOD OF EFFLUENT. CAT WAS DEAD OF ASPHYXIATION AND CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS HAD BEEN LIGATED. ASSAY OF SPASMOGEN WAS PERFORMED ON ISOLATED RAT ILEAL STRIP.

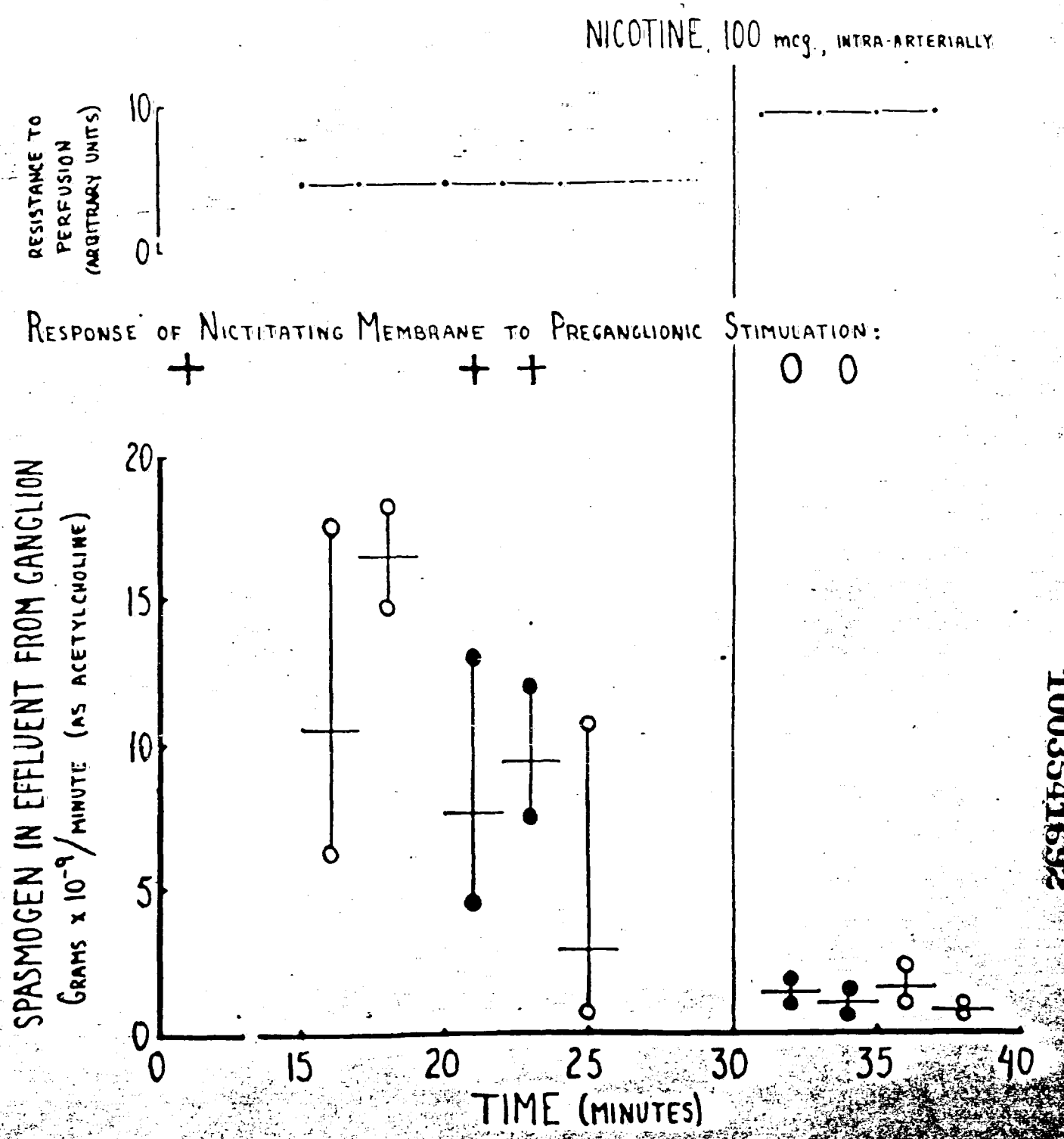

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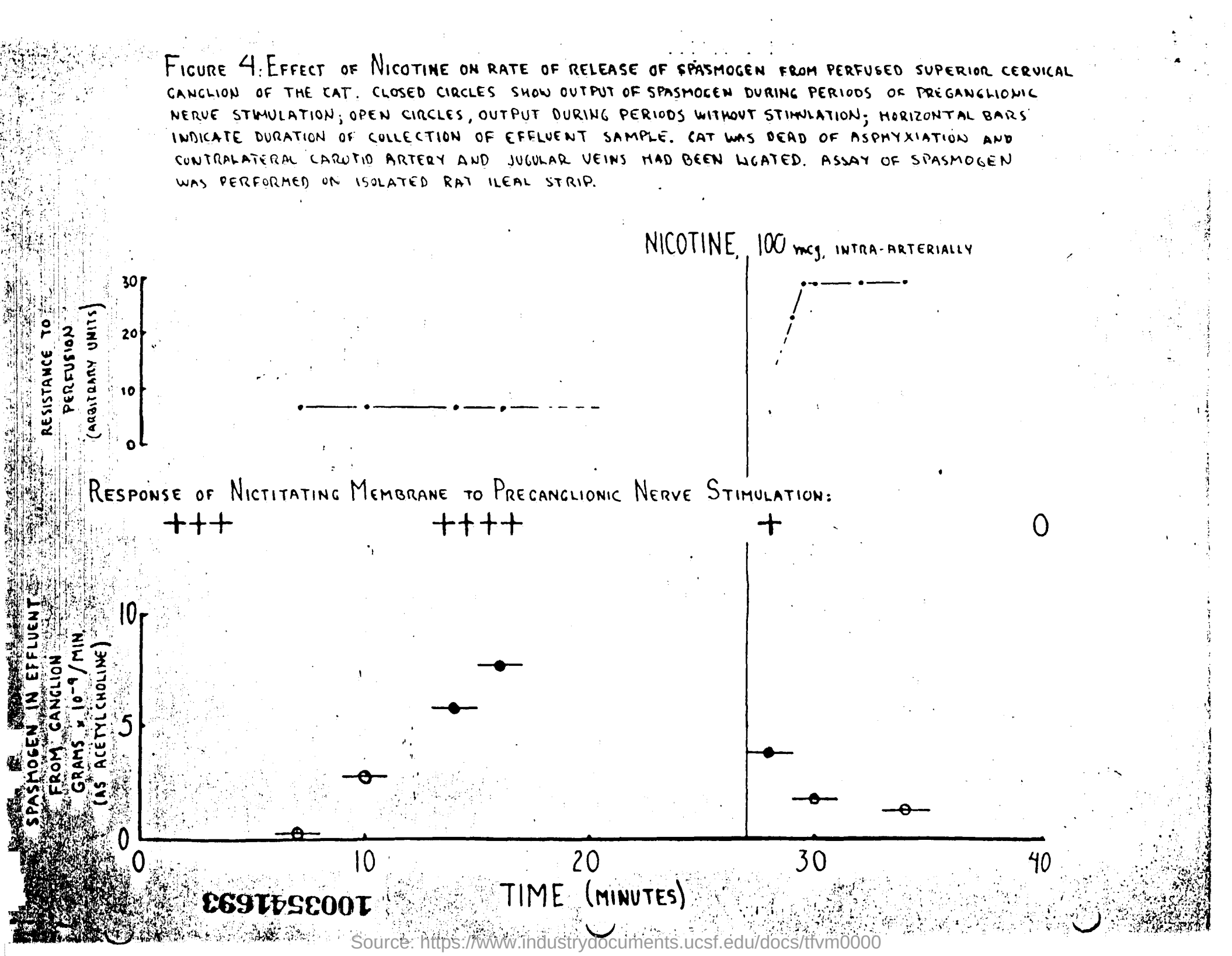
FIGURE 4. EFFECT OF NICOTINE ON RATE OF RELEASE OF SPASMOGEN FROM PERFUSED SUPERIOR CERVICAL GANGLION OF THE CAT. CLOSED CIRCLES SHOW OUTPUT OF SPASMOGEN DURING PERIODS OF PREGANGLIONIC NERVE STIMULATION; OPEN CIRCLES, OUTPUT DURING PERIODS WITHOUT STIMULATION; HORIZONTAL BARS INDICATE DURATION OF COLLECTION OF EFFLUENT SAMPLE. CAT WAS DEAD OF ASPHYXIATION AND CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS HAD BEEN LIGATED. ASSAY OF SPASMOGEN WAS PERFORMED ON ISOLATED RAT ILEAL STRIP.



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Comm.
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#170R2

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

(Cf. #170 - Activated: 2/1/58
Renewed: 2/1/'59)

Renewal
Application for Research Grant

Date: December 8, 1959

1. Name of Investigator: Edward W. Pelikan, M.D.

2. Title: Associate Professor of Pharmacology

3. Institution & Address: Department of Pharmacology and Experimental Therapeutics
Boston University School of Medicine
80 East Concord Street
Boston 18, Massachusetts

4. Project or Subject: Pharmacological analysis of the mechanism by which nicotine impairs transmission through autonomic ganglia.

5. Detailed Plan of Procedure: In previous reports of the present project, we described the evidence which lead us to believe that nicotine prevents transmission of nerve impulses through autonomic ganglia by a mechanism hitherto never ascribed to nicotine. In brief, we believe that nicotine can modify cardiovascular and other autonomic effector activity by preventing release of acetylcholine from nerve endings in autonomic ganglia, and thereby prevent nerve impulse transmission. We have not attempted to investigate the mechanism by which nicotine causes stimulation of autonomic ganglia.

The data upon which this hypothesis is based were obtained by recording the mechanical responses of the cat nictitating membrane to high and low frequencies of preganglionic nerve stimulation. These data were compared with mathematical formulations of effects to be expected were a hypothetical drug to impair ganglionic transmission by impairing acetylcholine release or, alternatively, by preventing access of acetylcholine to postsynaptic cell receptors. The excellent fit of the data obtained with nicotine, tetraethylammonium ions, morphine, and hexamethonium ions to the theoretical formulations led to our establishing the hypothesis summarized above.

During the past year the over-all object of our work has been to obtain direct experimental evidence that impaired ganglionic transmission produced by nicotine, tetraethylammonium or morphine was accompanied by a decrease in the rate of release of acetylcholine from the ganglion, and that hexamethonium blocked ganglionic transmission without affecting acetylcholine release. The work was performed in two parts:

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(1) A method was developed, using the isolated rat ileal strip, which permitted the bioassay of quantities of acetylcholine as low as one one-thousandth of a microgram. The assays could be performed without interference by the presence of nicotine, tetraethylammonium, or hexamethonium in quantities one thousand to one hundred thousand times as large. Using this technique it was possible to demonstrate that nicotine in concentrations of 10 mcg/ml completely suppressed the release of acetylcholine from isolated guinea pig ileal segments; no such suppression of acetylcholine release was obtained with concentrations of tetraethylammonium or hexamethonium as large as 1 mg/ml. A summary of this work was submitted to the Scientific Advisory Board on May 7, 1959.

(2) The superior cervical ganglion of the anesthetized cat was isolated and perfused with Ringer's solution using, essentially, the method of Kibjakov. Using this technique, the ganglion and its nerve supply, and the nerve supply to the nictitating membrane remain functional for reasonably long periods of time. The effluent from the ganglion was collected before, during, and after various procedures, including the injection of nicotine, and the acetylcholine content of each sample was assayed using the bioassay technique described above. A brief summary of the experimental results is appended to this application; a detailed summary will be submitted upon the expiration of the current research grant.

We propose to continue our research by extending our investigations of the effects of nicotine and other ganglionic "blocking" agents on the output of acetylcholine from the isolated, perfused superior cervical ganglion of the cat. Specifically, at least four kinds of experiments need to be performed:

1. Additional experiments must be performed to confirm the observed effects of nicotine in decreasing the output of acetylcholine from the ganglion. At the present time, satisfactory experiments with nicotine have been performed in three cats; for only two experiments could the results of the bioassay be interpreted unequivocally. For both experiments, the data indicate that acetylcholine output from the ganglion was decreased abruptly and remarkably at the time ganglionic transmission was prevented by the injected nicotine.

2. Experiments must be performed with agents such as morphine and tetraethylammonium, which we anticipate will prevent ganglionic transmission and decrease acetylcholine output as nicotine seems to do. Experiments must be performed using hexamethonium to produce ganglionic blockade; we anticipate such a blockade to be unaccompanied by significant changes in acetylcholine release. In one experiment performed so far with hexamethonium, it was found that the acetylcholine content of the effluent from the ganglion was too low, even before drug administration, to permit reliable assays.

   It is obviously crucial to our hypothesis concerning the mechanism of action of nicotine in preventing ganglionic transmission that we demonstrate that agents which affect the response of the nictitating membrane to high and low frequency stimulation, as nicotine does, also have effects on acetylcholine output like those of nicotine. Similarly, a counter proof is required; hexamethonium and nicotine affect the response of the membrane differently, and we must demonstrate different effects of the two on acetylcholine output.

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3. Experiments must be performed to determine the significance of changes in vascularity in the perfused tissues in altering the quantity of acetylcholine in the effluent. In contrast to our expectation based on a study of the literature, we have found it impossible, thus far, to isolate the afferent and efferent vascular supply of the ganglion from the systemic circulation, or from the circulation to other tissues which may act as potential sources at least of acetylcholine. Furthermore, we have observed that injection of nicotine to the ganglion causes changes in resistance to perfusion and changes in the fraction of infused fluid recovered as effluent; these results may indicate that acetylcholine detected in "control" samples of effluent may be derived in whole or in part from areas of the cat quite different from those which supply the acetylcholine which is detected in effluent collected during the course of the nicotine effect.

   The effect of experimental manipulations in changing the areas of perfusion and drainage in the Kibjakov preparation and the consequent effects of such changes on the apparent output of "transmitter" from the "ganglion" seems not to have been discussed in previous publications. This is most remarkable since the theory of acetylcholine as the neurohumoral transmitter in ganglia rests in part on the inference that changes in acetylcholine output from the "ganglion" are related only to the functional state of pre-ganglionic nerve terminals in the ganglion alone.

   We hope to investigate the relationship between the vascularity of the perfused area and acetylcholine output in experiments in which:

   a. We study the effects of surgical extirpation of the ganglion or pre- and post-ganglionic nerve section on acetylcholine output and resistance to perfusion.

   b. Vasoconstriction in the perfused area is produced by a substance such as vasopressin which has no known effects on acetylcholine release.

   c. An attempt is made to influence acetylcholine output with agents such as morphine which may well be given without producing significant effects on the vasculature. Any effects of morphine on acetylcholine output may reasonably be expected to outlast effects on vessels such as might be produced by histamine release.

4. Experiments should be performed to identify the spasmogen assayed in our rat ileal preparation with acetylcholine. Like the activity of acetylcholine, the activity of the spasmogen we assay as acetylcholine is destroyed by boiling of the effluent or prevented by atropinization of the ileum. However, in our 4-point-assay design, dose-effect curves for the spasmogen have slopes consistently higher than the slopes of the curves for the standard, acetylcholine. According to the logic of bioassay, this indicates that the spasmogenic activity of the samples does not reside in a molecular species identical with acetylcholine. Previous investigators have routinely used 3-point-assays of the effluent to

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detect "acetylcholine." Such a technique permits the use of small effluent volumes and permits a larger proportion of "successful" assays and experiments, but precludes identification of the standard with the unknown agent.

Resolution of the problem of the non-parallelism of the dose-effect curves of the standards and unknown agents may take two courses:

a. Alteration of the experimental design--by using other anesthetic agents, anti-coagulant drugs, or anti-cholinesterase drugs in the perfusing fluid--to determine whether the lack of parallelism is adventitious.

b. Concentration of the effluent and an attempt to identify acetylcholine in it by chemical or enzymatic means.

The methods presently available to us seem adequate to determining whether administration of nicotine, or other agents which impair ganglionic transmission, can alter both ganglionic transmission and "acetylcholine" output in the effluent. An adequate biological interpretation of these facts, an attempt to determine causal relations between them, requires further identification of the ganglion as the only significant source of the spasmogen assayed and more certain identification of the spasmogen with acetylcholine.

6. Budget Plan:

Salaries	$ 4,500. *
Expendable Supplies	2,000.
Permanent Equipment	2,000.
Overhead	1,365.
Other	600.
Total	$ 10,465.

* E. W. Pelikan $1,000.
Research Asst. 3,500.

7. Anticipated Duration of Work:

Two years; the budget plan above applies only to the first year of the research.

8. Facilities and Staff Available:

Facilities and staff of the Department of Pharmacology and Experimental Therapeutics of the Boston University School of Medicine. The staff members are competent and well equipped to aid the course of this study, especially insofar as special knowledge of biochemical and physical chemical methods might be required, and insofar as special techniques might be required to record and study cardiovascular phenomena. No salaries are requested for persons who might aid the work in this way.

9. Additional Requirements:

None.

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10. Additional Information (Including relation of work to other projects and other sources of supply):

This proposed research program is the direct outgrowth and continuation of research now being conducted by the applicant under a grant from the Tobacco Industry Research Committee. The work is also an extension of previous investigation by the applicant; these investigations have included studies of the pharmacology of the neuromuscular junction in several species, studies of the structure-activity relationships among neuromuscular blocking agents, and studies of the actions of nicotine and sympathomimetic amines on isolated intestinal strips, the frog rectus abdominis muscle, and on the superior cervical ganglion of the cat.

Application for support of this work has not been made to any other agencies.

The applicant will spend about three-fourths of his time on these investigations.

/s/ Edward W. Pelikan

/s/ K. M. Haetzfeld

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TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #170

Report No. 1

Edward W. Pelikan, M.D.
Boston University School of Medicine

September, 1958

A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms

Experiments were performed to determine the effects of nicotine, and several other agents which block ganglionic transmission, on the responses of the cat nictitating membrane to stimulation of the preganglionic nerve fibers at both low and high rates of stimulation.

Methods: Experiments were performed on 25 adult cats of both sexes, which had been anesthetized with sodium pentobarbital (35 mg./K., intraperitoneally). In each experiment, the trachea was cannulated, and the animal breathed spontaneously throughout the experiment. Femoral blood pressure was recorded kymographically with a mercury manometer; intravenous injections were made via a femoral vein catheter. A stainless steel cannula was passed retrograde into the right lingual artery; drug injections through the cannula could be directed toward the head or toward the ipsilateral superior cervical ganglion by occluding the common carotid artery below or above, respectively, the origin of the lingual artery. Square wave stimuli of 1.0 msec. duration and of supramaximal voltage were applied from a Grass S4C stimulator via shielded platinum electrodes to the cephalic end of the cut preganglionic cervical sympathetic trunk on the right side. Contractions of the right nictitating membrane were recorded kymographically by means of an isotonic lever system of 7-fold magnification; tension on the nictitating membrane was 3.0 Gm.

In each experiment, trains of stimuli were applied to the preganglionic fibers for periods of 20 seconds every 2 to 3 minutes, specifically, as soon after a train of stimuli had been given as the length of the nictitating membrane returned to a steady level. During the first 10 seconds of each period of stimulation a frequency of stimulation of 1.25 stimuli/second was used; during the second half of each period a frequency of 20 stimuli/second was used.

For each train of stimuli the maximum degree of contraction of the nictitating membrane during the periods of low and high frequency stimulation, respectively, were determined from the kymograph record by measuring the maximum deviation of the writing lever, during the periods of low and high frequency stimulation, from the base-line record of nictitating membrane length recorded before the onset of the train of stimuli. The degree of contraction of the membrane at the end of the period of high frequency stimulation ("high frequency response";HFR) was always greater, under control conditions, than the degree of contraction at the end of the period of low frequency stimulation ("low frequency response";LFR). In control experiments it was determined that the same HFR was produced whether or not an LFR preceded it.

In each experiment trains of stimuli were delivered to the preganglionic fibers at intervals, as described above, until constant HFR's and LFR's were produced. Then a preselected dose of one of the drugs under investigation was administered to the ganglion via the lingual artery cannula. One minute after the end of the injection, a train of stimuli was applied to the nerve, and trains of stimuli were then applied successively as described above. When the HFR and LFR were judged to have returned to a stable level, a

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second drug dose was given, and the sequence of trains of stimuli was repeated. Care was taken to avoid interaction of the effects of successive drug doses. Most of the cats studied received more than one drug.

The drugs which have been studied thus far are nicotine, tetraethylammonium bromide (TEA), hexamethonium chloride (C6), and morphine sulfate. The drugs were prepared as stock solutions in 0.9% sodium chloride solution, and the pH's of the solutions were adjusted to 7.4 with hydrochloric acid and sodium hydroxide. Dilutions have been prepared from stock solutions, and buffered to pH 7.4, as they have been needed. Drug solutions were refrigerated between periods of use, but were allowed to reach room temperature before being used. Control injections of buffered 0.9% sodium chloride solution, at room temperature, have consistently failed to alter either the HFR or LFR.

Results: All of the drugs which were tested produced at some dose level a decrease in the response of the nictitating membrane to preganglionic stimulation. Data in the literature indicate that failure of ganglionic transmission, rather than failure of nerve conduction, neuro-effector transmission, or effector activity is responsible for this decreased response in the case of all of the agents we have studied. With effective doses of C6 (20-120 mcgm., intraarterially*), TEA (200-1000 mcgm., i.a.) and morphine (100-400 mcgm., i.a.) the only effects observed were decreases in the response of the nictitating membrane; contraction of the membrane has not been produced by any of these agents. Nicotine, in doses larger than 2-3 micrograms, invariably produced contraction of the membrane when the dose was directed toward the ganglion, but never produced contraction when the injected mass was directed toward the head and membrane itself. In general, the magnitude of the contraction produced by nicotine was proportional to the dose of nicotine used; maximal contractions were produced with doses of 20-80 micrograms. Low doses of nicotine (10-15 mcgm. or less, i.a.) occasionally facilitated the HFR's and LFR's invoked immediately after relaxation of the membrane after the nicotine-induced contraction. Larger doses of nicotine (20-80 mcgm., i.a.) regularly caused a decreased response of the membrane to preganglionic stimulation. Both the facilitatory and inhibitory effects of nicotine on the HFR and LFR were, in general, proportional to the dose which produced them.

All of the drug effects which we have observed were completely reversible. With all of the drugs studied, characteristic effects on HFR and LFR were observed after doses which produced no alteration in systemic blood pressure. With the larger doses, particularly of TEA and nicotine, alteration in the response of the membrane was frequently accompanied in time by changes of blood pressure such as are usually produced by smaller doses of the drugs given intravenously.

With regard to their ganglionic "blocking" effects, nicotine, TEA, and morphine and C6 differed in their effects on HFR and LFR, as determined at the times of maximal drug effect. Nicotine, TEA, and morphine depressed the LFR relatively more than they did the HFR. In contrast, as Paton and Zaimis observed, C6 depressed the HFR relatively more than the LFR. These differential effects of the drugs can be demonstrated by comparing, for the different drugs, the change produced in HFR (HFR at time of maximal effect/HFR before drug administration; HFR_a/HFR_b) with the change produced in the corresponding LFR (LFR at time of maximal effect/LFR before drug administration; LFR_a/LFR_b). A

* Dose ranges include the minimally and maximally effective doses studied in all experiments.

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demonstration of this kind, of results obtained after all doses, in all experiments with nicotine, TEA and C6 is given in the table:

DRUG	No. of Cats	No. of Injections	No. of Effective Injections*	INCIDENCE OF RESPONSES $HFR_a/HFR_b > LFR_a/LFR_b$	$HFR_a/HFR_b = LFR_a/LFR_b$	$HFR_a/HFR_b < LFR_a/LFR_b$
Nicotine	8	45	30	21	2	7
TEA	10	52	51	42	0	9
Morphine	2	4	4	4	0	0
C6	7	29	29	7	0	22

* Injections were considered to be effective when the LFR was decreased by the drug.

Comparison of the tabulated pattern of responses for nicotine and C6 and for TEA and C6 by the method of Chi-square indicated that the patterns for nicotine and C6 (Chi-square = 12.7) and TEA and C6 (Chi-square = 21.7) would occur much less than once in a hundred times on the basis of random sampling from a single population of responses. In contrast, comparing the pattern of responses for nicotine and TEA indicated (Chi-square = 0.24) that such a pattern would be observed at least six times out of ten if responses were drawn randomly from a single population. We infer that nicotine and TEA produce qualitatively similar patterns of alteration in HFR and LFR, which differ from that produced by C6, and that nicotine and TEA may have similar mechanisms of action on ganglionic transmission which may differ from that of C6. Too few experiments have been performed with morphine to permit meaningful analysis by the method of Chi-square. Data obtained with morphine indicate its pattern of responses is similar to that produced by nicotine and TEA.

Rosenblueth demonstrated that partial transection of preganglionic fibers to the superior cervical ganglion distal to a stimulating electrode (i.e., decreasing the quantity of neurotransmitter released for each stimulus without decreasing the amount of transmitter released per neurone activated) caused a greater decrease in the LFR of the nictitating membrane than in the HFR. Paton, and Schaumann, concluded that morphine (which in our preparation affects LFR more than HFR) caused a decrease in the release of acetylcholine (ACh) from the myenteric plexus of the guinea pig. Paton and Zaimis observed that C6 blocks ganglionic transmission and the response to exogenous ACh without altering the release of ACh from the ganglion, and that C6 affects the HFR more than the LFR. Paton and Zaimis concluded that C6 acts at the ganglion by decreasing the number of receptors on the post-synaptic cell bodies available to ACh released in normal amounts from the pre-synaptic nerve terminals. Considering our results with nicotine, TEA, and morphine in the light of these observations lead us to the hypothesis that nicotine, TEA, and morphine might decrease ganglionic transmission by decreasing the release of ACh from the pre-synaptic nerve terminals, and that the action of these compounds might differ qualitatively from those of C6, which we believe acts in the manner suggested by Paton and Zaimis.

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To test quantitatively the degree to which our data fitted this hypothesis, the following procedure was carried out:

1. We determined that with our recording system, magnitude of contraction of the nictitating membrane was linearly related to the logarithm of the frequency of supramaximal stimuli applied to the preganglionic fibers. The curve passed through the points: (20 stimuli/second, 100% response), (2.5 stimuli/second, 50% response).

2. The following assumptions were made:

a. The amount of ACh released from pre-synaptic nerve terminals per unit time is a linear function of stimulus frequency; ACh release at a frequency of 20 stimuli/second was arbitrarily considered to equal 100 Units, and at a frequency of 1.25 stimuli/second, to be 6.25 Units.

b. The per cent of the total available receptors on the post-synaptic cell bodies occupied by ACh is a logarithmic function of the ratio: (ACh Units released per unit time/total number of receptors available).

c. The magnitude of contraction of the nictitating membrane is a linear function of the per cent of the available receptors occupied by ACh.

d. Drugs may affect ganglionic transmission either by decreasing the release of ACh or by making receptors unavailable to ACh, or by both mechanisms. (We recognize, but have not included in our theoretical analysis, the possibility of influencing ganglionic transmission by other means too, for example, by altering the rate of hydrolysis of ACh.)

3. Computations were made which yielded theoretical magnitudes of HFR and LFR under control conditions and when hypothetical decreases of varying amounts were made in ACh output per nerve volley or in the number of post-synaptic receptors available to ACh.

4. Curves were plotted, each of which showed the theoretical relationship between HFR and LFR before and after the administration of drugs which decreased ganglionic transmission by one of the two mechanisms hypothecated in "d." above. On these curves, the abscissal unit was LFR_a/LFR_b, and the ordinal unit was $(HFR_a/LFR_a) / (HFR_b/LFR_b)$. These units are dimensionless and permit combining all the data obtained with a given drug in one or more experiments on a single graph. The metameter of dose is eliminated from data plotted in this way. The theoretical curves are presented in Figure 1.

5. For each drug studied, the ratios described in "4." above were computed for responses to all stimulus trains, for all drug doses in all experiments. It should be noted that ratios were computed for all responses elicited during the entire course of action of each drug dose. The experimental data were plotted as were the theoretical ratios described above. Typical data are presented in Figures 2, 3, and 4.

It can be seen by inspection of Figures 2 and 3 that data obtained from experiments with nicotine and TEA fit closely to the theoretical curves obtained using the assumption that decreased ganglionic transmission was effected only by

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decreasing ACh output from pre-synaptic nerve terminals. Inspection of Figure 4, on the other hand, indicates that data obtained from experiments with C6 fit the theoretical curve plotted using the assumption that decreased ganglionic transmission was produced only by decreasing the number of receptors available to ACh released in normal quantities from the pre-synaptic nerve terminals. However, it is obvious that more data must be collected before the precise fit of data for C6 to the theoretical curve can be determined.

Conclusions: Our data are consistent with our hypothesis that nicotine, TEA, and morphine, under the conditions of our experiments, decrease transmission through the superior cervical ganglion of the cat by interfering with the release of ACh from pre-ganglionic nerve terminals. Our data support the view that the ganglionic effect of C6 is produced by making receptors on the post-synaptic cell body unavailable to ACh released in normal quantities.

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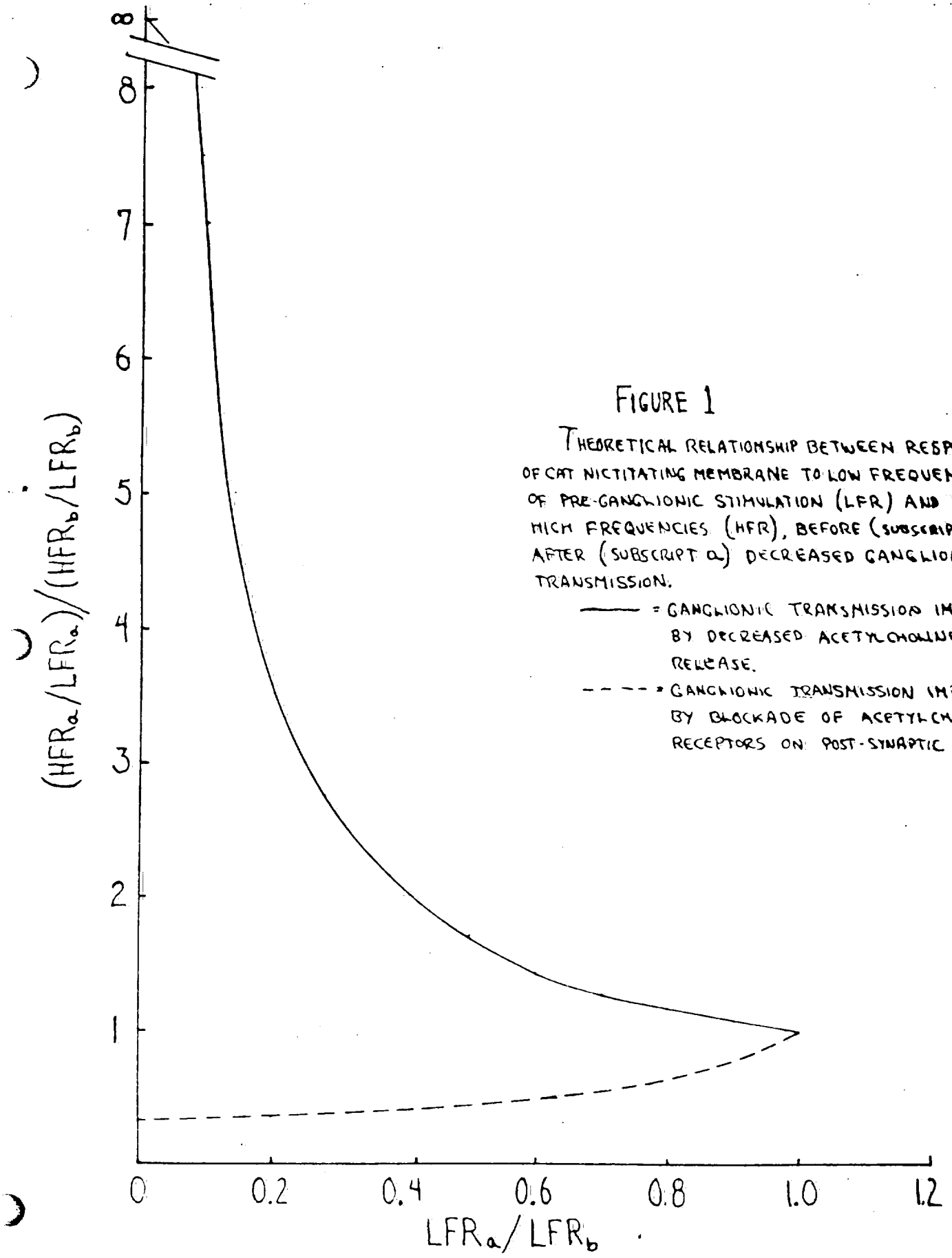

FIGURE 1

THEORETICAL RELATIONSHIP BETWEEN RESPONSE OF CAT NICTITATING MEMBRANE TO LOW FREQUENCIES OF PRE-GANGLIONIC STIMULATION (LFR) AND TO HIGH FREQUENCIES (HFR), BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) DECREASED GANGLIONIC TRANSMISSION.

——— = GANGLIONIC TRANSMISSION IMPAIRED BY DECREASED ACETYLCHOLINE RELEASE.

- - - - = GANGLIONIC TRANSMISSION IMPAIRED BY BLOCKADE OF ACETYLCHOLINE RECEPTORS ON POST-SYNAPTIC CELL

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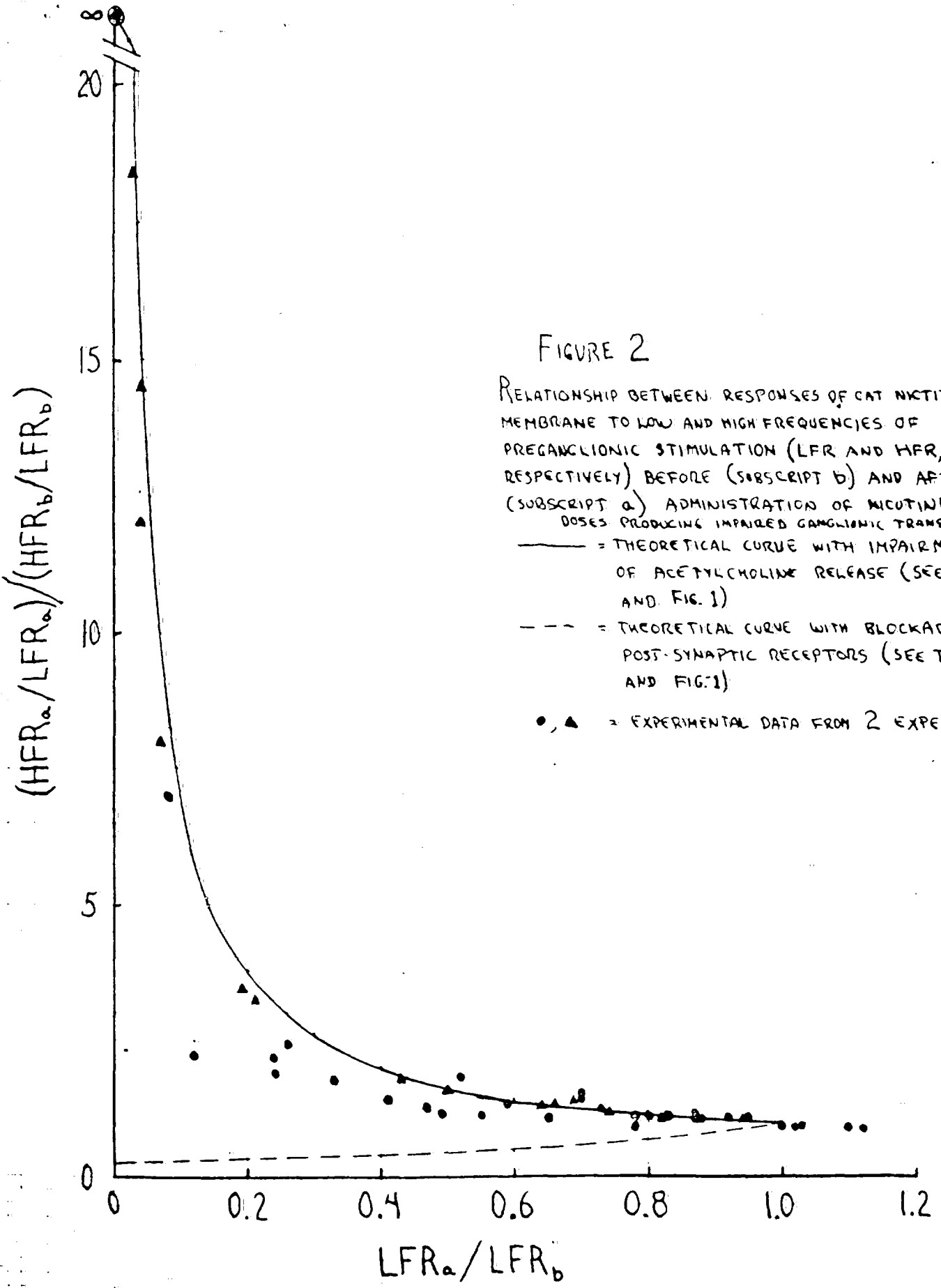

FIGURE 2

RELATIONSHIP BETWEEN RESPONSES OF CAT NICTITATING MEMBRANE TO LOW AND HIGH FREQUENCIES OF PREGANGLIONIC STIMULATION (LFR AND HFR, RESPECTIVELY) BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) ADMINISTRATION OF NICOTINE, IN DOSES PRODUCING IMPAIRED GANGLIONIC TRANSMISSION.

——— = THEORETICAL CURVE WITH IMPAIRMENT OF ACETYLCHOLINE RELEASE (SEE TEXT AND FIG. 1)

– – – = THEORETICAL CURVE WITH BLOCKADE OF POST-SYNAPTIC RECEPTORS (SEE TEXT AND FIG. 1)

●, ▲ = EXPERIMENTAL DATA FROM 2 EXPERIMENTS

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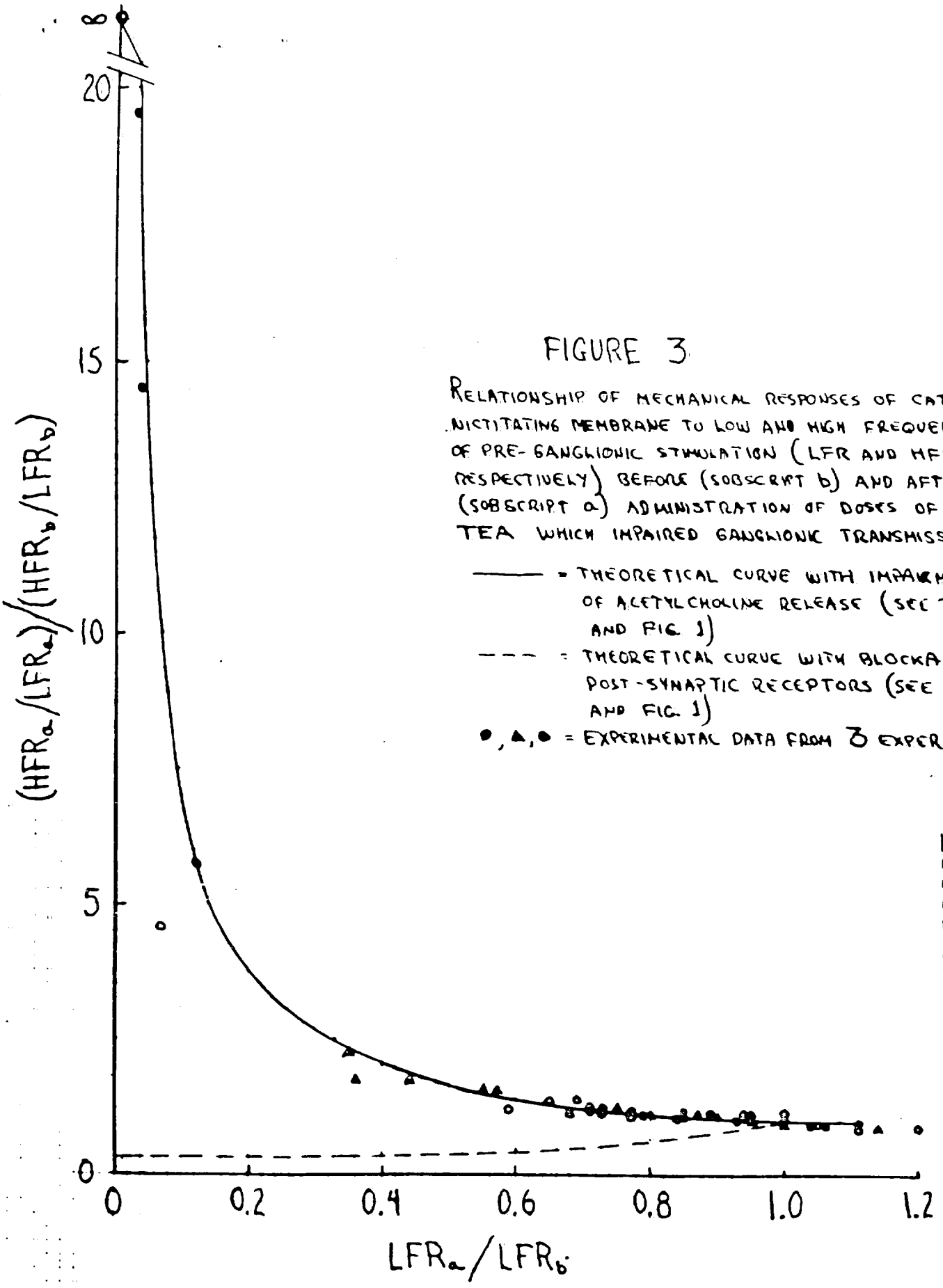

FIGURE 3

RELATIONSHIP OF MECHANICAL RESPONSES OF CAT NICTITATING MEMBRANE TO LOW AND HIGH FREQUENCIES OF PRE-GANGLIONIC STIMULATION (LFR AND HFR, RESPECTIVELY) BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) ADMINISTRATION OF DOSES OF TEA WHICH IMPAIRED GANGLIONIC TRANSMISSION.

——— = THEORETICAL CURVE WITH IMPAIRMENT OF ACETYLCHOLINE RELEASE (SEE TEXT AND FIG. 1)

– – – = THEORETICAL CURVE WITH BLOCKADE OF POST-SYNAPTIC RECEPTORS (SEE TEXT AND FIG. 1)

●, ▲, ● = EXPERIMENTAL DATA FROM 3 EXPERIMENTS

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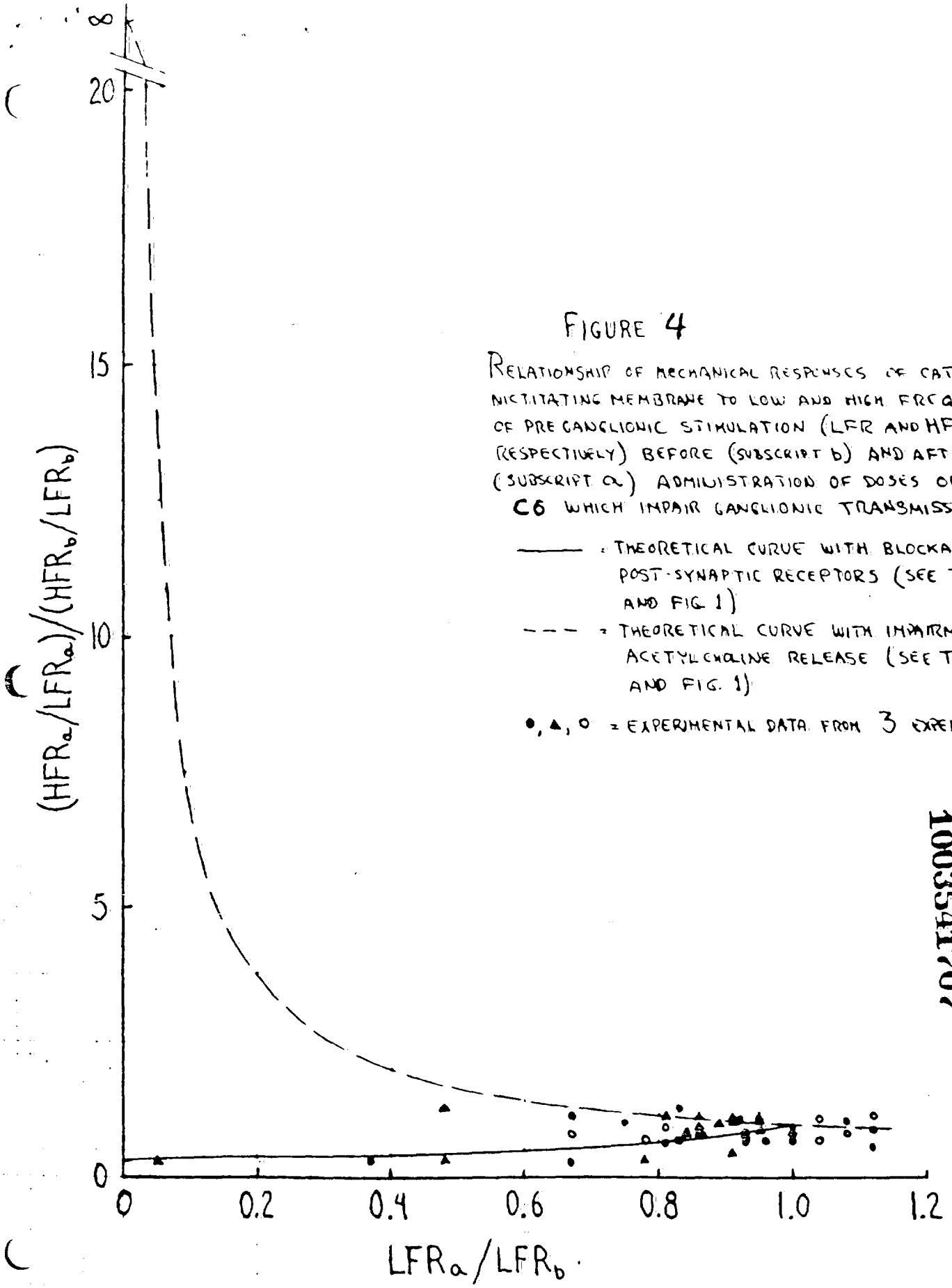

FIGURE 4

RELATIONSHIP OF MECHANICAL RESPONSES OF CAT NICTITATING MEMBRANE TO LOW AND HIGH FREQUENCIES OF PREGANGLIONIC STIMULATION (LFR AND HFR, RESPECTIVELY) BEFORE (SUBSCRIPT b) AND AFTER (SUBSCRIPT a) ADMINISTRATION OF DOSES OF C6 WHICH IMPAIR GANGLIONIC TRANSMISSION.

——— = THEORETICAL CURVE WITH BLOCKADE OF POST-SYNAPTIC RECEPTORS (SEE TEXT AND FIG. 1)

– – – = THEORETICAL CURVE WITH IMPAIRMENT OF ACETYLCHOLINE RELEASE (SEE TEXT AND FIG. 1)

•, ▲, ○ = EXPERIMENTAL DATA FROM 3 EXPERIMENTS

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#170R1
(Activated 2/1/58)

Application For Research Grant

Date: November 17, 1958

1. Name of Investigator:
Edward K. Pelikan, M.D.

2. Title:
Associate Professor of Pharmacology

3. Institution & Address:
Department of Pharmacology and Experimental Therapeutics
Boston University School of Medicine
80 East Concord Street, Boston 18, Massachusetts

4. Project or Subject:
Pharmacological analysis of the mechanism by which nicotine impairs transmission through autonomic ganglia.

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5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Although it is well known that cardiovascular function and other autonomic effector functions are profoundly affected by nicotine, little is known of precisely how nicotine acts on cells. Specifically, there is little experimental data concerning the mechanism by which nicotine decreases the function of the autonomic nervous system. Since the present project was begun, evidence has been obtained which indicates a new, potentially important, and previously unreported mode of action of nicotine in influencing transmission through autonomic ganglia.

Our data lead us to believe that nicotine, tetraethylammonium ions, and morphine may decrease transmission through autonomic ganglia by decreasing the amount of acetylcholine released per nerve volley from the terminal branches of preganglionic nerve fibers. This mechanism of action is in contrast to that of hexamethonium ions, which act, according to all available evidence, by decreasing the number of acetylcholine receptors available on post-synaptic nerve cell bodies.

Our experiments were performed using a cat nictitating membrane preparation. The mechanical responses of the membrane to high and low frequencies of preganglionic nerve stimulation were compared before and after intra-arterial injection to the ganglion of the drugs which decreased ganglionic transmission.

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The experimental results were compared with mathematical predictions of the results to be obtained during the actions of drugs which might prevent ganglionic transmission by decreasing acetylcholine output from preganglionic nerve terminals, or, alternatively, by preventing access of acetylcholine to post-synaptic cell receptors. The fit of the experimental data to the theoretical formulations was excellent and form the basis of the conclusions described above. A report of our work and a summary of our data was submitted to the Scientific Advisory Board on July 31, 1958.

We propose to continue our investigation by measuring acetylcholine output from ganglionic tissue, and to provide experimental evidence that nicotine, tetraethylammonium, and morphine can decrease this output. We hope to demonstrate that nicotine, tetraethylammonium, and morphine both decrease acetylcholine output and decrease ganglionic transmission at similar dose levels appropriate to the respective agents. We hope to demonstrate, furthermore, that concentrations of the drugs greater than those required to decrease acetylcholine release and ganglionic transmission are needed to prevent the effects of acetylcholine.

We propose to carry out our analysis of the actions of nicotine, tetraethylammonium (TEA), morphine, and hexamethonium (C6) by means of the following sequence of investigations:

1. To determine whether the agents can prevent release of acetylcholine from autonomic preganglionic nerve terminals:

a. An assay method must be developed which will permit detection and assay of minute quantities of acetylcholine in the presence of interfering substances such as nicotine (which has actions similar to acetylcholine in most bioassay preparations) or TEA and C6 (which interfere with many of the chemical methods of determining acetylcholine). Preliminary experiments have indicated that a successful bioassay of acetylcholine, in dilutions as low as 10^{-9} to 10^{-8}, can be carried out using the isolated eserinized rat ileum. This tissue is relatively insensitive to nicotine and, because of its low level of spontaneous tone, is not affected by TEA or C6. Increased specificity of the assay can probably be obtained by using differential pharmacological blockade of other substances, such as histamine or serotonin, which might appear in tissue extracts or the efflux from ganglionated tissues. We are actively pursuing the development of this bioassay technique, under the terms of our present grant.

b. It must be determined whether, in fact, nicotine, TEA, and hexamethonium can decrease acetylcholine output from some nervous tissue, the acetylcholine output of which is known to be decreased by some drug. Schaumann and, independently, Paton have demonstrated that morphine can in fact decrease acetylcholine output from excised guinea pig intestine. We propose to confirm the findings of Schaumann and Paton using the bioassay method described above (as a validation of our bioassay method) and then determine whether our validated method indicates that nicotine, TEA and heaxamethonium can or cannot influence acetylcholine release.

c. It must be determined whether nicotine, TEA, and hexamethonium can influence the acetylcholine output from the cat superior cervical ganglion stimulated via its preganglionic fibers. Two experimental methods are available for pursuit of this part of the problem: 1. The collection of venous effluent from the ganglion in situ and the estimate of the acetylcholine content of the effluent in the presence and absence of the drugs under consideration. Elaboration of this

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technique, as it has been used by MacIntosh and others, might well permit simultaneous recording of the responses of the nictitating membrane to preganglionic stimulation; such an elaboration might be the perfusion of the nictitating membrane ipsilateral to the test ganglion with blood from the contralateral carotid artery. 2. The collection of the medium in which an isolated ganglion might be suspended with its preganglionic innervation, and the determination of the acetylcholine content of the medium during preganglionic stimulation, in the presence and absence of the test drugs.

2. To determine whether agents which decrease ganglionic transmission and can decrease acetylcholine output from preganglionic nerve terminals have these effects in similar dose ranges and block the effects of acetylcholine only in higher dose ranges:

a. Nicotine, TEA, and morphine can block the effects on ganglia of injected acetylcholine; no studies have been made which compare precisely the doses required to produce impaired ganglionic transmission and those which block acetylcholine. We believe that such a study might be made in a preparation of both nictitating membranes of a cat, in one of which responses are induced by preganglionic nerve stimulation, and in the other of which responses are induced by close intra-arterial injection to the ganglion of acetylcholine. After systemic, i.e., intravenous, administration of the test drugs, the abilities of the agents to prevent ganglionic transmission and to block acetylcholine might be compared directly and quantitatively.

b. If attempts to demonstrate the effects of nicotine, TEA, and hexamethonium on acetylcholine release from ganglia in vitro are successful, direct comparisons of the concentrations of test drug required to impair ganglionic transmission and those required to decrease acetylcholine output might be made in vitro. In this case, the measure of decreased ganglionic transmission might be the decrease in amplitude of post-ganglionic action potentials displayed oscillographically. Our laboratory is equipped with amplifiers and oscilloscopes necessary for this work; it would, however, be necessary to purchase a kymographic camera capable of recording post-ganglionic responses to high frequency preganglionic stimulation.

If the results of the experiments we propose provide confirmation of our hypothesis of the mechanism of action of nicotine in decreasing transmission in autonomic ganglia, we hope to investigate whether such a mechanism might be responsible for the actions of nicotine at other sites such as the skeletal neuromuscular junction, and in the central nervous system.

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6. Budget Plan:

Salaries	$4,500*
Expendable Supplies	2,000
Permanent Equipment	2,000
Overhead (at 15%)	1,365
Other	600
Total	$10,465

* E.W. Pelikan $1,000
Research Assistant $3,500

7. Anticipated Duration of Work:

Two years; the budget plan above applies only to the first year of the research.

8. Facilities and Staff Available:

Facilities and staff of the Department of Pharmacology and Experimental Therapeutics of the Boston University School of Medicine. The staff members are competent and well equipped to aid the course of this study, especially insofar as special knowledge of biochemical and physical chemical methods might be required, and insofar as special techniques might be required to record and study cardiovascular phenomena. No salaries are requested for persons who might aid the work in this way.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

This proposed research program is the direct outgrowth and continuation of research now being conducted by the applicant under a grant from the Tobacco Industry Research Committee. The work is also an extension of previous investigation by the applicant; these investigations have included studies of the pharmacology of the neuromuscular junction in several species, studies of the structure-activity relationships among neuromuscular blocking agents, and studies of the actions of nicotine and sympathomimetic amines on isolated intestinal strips, the frog rectus abdominis muscle, and on the superior cervical ganglion of the cat.

Application for support of this work has not been made to any other agencies.

The applicant will spend about three-fourths of his time on these investigations.

Signature /s/ Edward W. Pelikan, M.D.
Director of Project

/s/ Russell V. Mahurin
Business Officer of the Institution

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

Date: June 28, 1957

1. Name of Investigator: Edward W. Pelikan, M.D.

2. Title: Associate Professor of Pharmacology

3. Institution & Address: Department of Pharmacology and Experimental Therapeutics
Boston University School of Medicine
80 East Concord Street, Boston 18, Massachusetts

4. Project or Subject: A study of structure-activity relationships among drugs which affect nicotine-sensitive physiological mechanisms.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

A. Introduction.

1. Although the gross pharmacological actions of nicotine have been described in detail, there is a remarkable lack of knowledge of how and why nicotine affects the cells it does. We wish to study the mechanisms by which nicotine acts and to attempt to learn what aspects of the chemical structure of nicotine are responsible for each of its major actions. We believe this study can best be pursued by a comparative investigation of the actions of drugs with a wide range of chemical structures, all of which drugs possess actions in common with nicotine. In this study, the known actions of nicotine will be used as a standard for comparison; the biological systems on which nicotine acts will function as indicator systems; the major experimental variable will be variation in chemical structure of a series of drugs.

2. The understanding of the effects of drugs on autonomic and somatic nervous system activity has been confused by the relative non-selectivity of action of agents, the great selectivity of action of which has been assumed. We intend to use the lack of selectivity of these agents as the factor crucial to our experiments.

a. It is well known that nicotine is a compound with little selectivity and specificity of action. It is known that in the reflex arc, nicotine may stimulate sensory receptors, modify the integrative action of the central nervous system, influence ganglionic transmission, and alter the ac-

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tivity of effector systems (at least in the case of skeletal muscle). Nicotine also has direct effects on the adrenal medulla and on isolated chromaffin masses, and on the posterior pituitary gland to cause the release into the circulation of substances which can affect a wide variety of tissues. The effects of nicotine are further complicated and made difficult to interpret since:

1. Nicotine given in large doses may diminish or abolish at least some of those activities stimulated by smaller doses.

2. Tachyphylaxis is developed toward at least some of the actions of nicotine.

3. Alteration of the state of the organism by any drug, including nicotine, induces compensatory reflex activity.

4. Even in a single tissue nicotine, present in a given concentration, may modify several functions of the tissue in opposite ways. Taugner, for example, has shown that the same dose of nicotine which will facilitate the homolateral flexion reflex of the decerebrate cat will inhibit the patellar reflex.

b. The relative lack of selectivity of action of some of the "autonomic drugs" is less well known and less well studied than is true for nicotine. It is well known that acetylcholine has both "nicotinic" and "muscarinic" effects, and that even muscarine has some "nicotinic" effects. Drugs used to produce ganglionic blockade are known to have variable amounts of activity at the neuromuscular junction, and in the central nervous system (Chen); at both of these sites ganglionic blocking drugs act antagonistically to nicotine.

Sympathomimetic amines have actions at at least four sites which are also sensitive to nicotine: Sympathomimetic amines can stimulate receptors in the area of the carotid body (Eyck and Comroe), can have effects on the central nervous system, can modify ganglionic transmission (Matthews, Trendelenburg, and others), and can alter the activity of effector organs, including skeletal muscle (Huidobro, and others).

We have already reported the results of our comparison of the effects of nicotine and a series of sympathomimetic amines on the isolated rabbit jejunum and the isolated frog rectus abdominis muscle. It was found that nicotine and derivatives of phenethylamine and tyramine had similar actions on the jejunal strips; in general, derivatives of phenethylamine and tyramine manifested their sympathomimetic actions only after the strips had been treated with a ganglionic blocking agent in a concentration sufficient to abolish the actions of nicotine. It could not be determined with certainty that epinephrine, norepinephrine and 3-hydroxytyramine had nicotine-like actions in this tissue. In the isolated frog rectus abdominis preparation, phenethylamine and tyramine derivatives had actions qualitatively like those of nicotine: all the compounds produced contracture of the muscle which could be prevented by tubocurarine, and tachyphylaxis developed to the actions of all of the agents. Epinephrine, norepinephrine and 3-hydroxytyramine failed to produce contracture of the frog muscle, but, rather, blocked the actions of nicotine on the muscle.

In our studies using the superior cervical ganglion of the cat, we have observed that all of a series of sympathomimetic amines had effects qualitatively similar to those of nicotine in inhibiting ganglionic transmission. The sympathomimetic amines most potent in this respect were those which also possessed the greatest sympathomimetic effect: epinephrine was three times as potent as nicotine in producing ganglionic blockade. Under these conditions

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of our experiments none of the agents, including nicotine, facilitated ganglionic transmission. Matthews, who used a different experimental procedure, found that at least the sympathomimetic amine, isopropylnorepinephrine, could produce such facilitation. Kewitz and Reinert used still another experimental method and found that epinephrine blocked ganglionic transmission and facilitated the ganglionic response to injected acetylcholine, but that amphetamine blocked the effects of acetylcholine and facilitated the effects of preganglionic nerve stimulation.

B. Specific proposal.

1. We propose to test the hypothesis that compounds with qualitatively similar actions on nicotine-sensitive mechanisms have chemical-structural features in common. We hope to identify such common chemical properties among nicotine, sympathomimetic amines, and ganglionic and neuromuscular blocking agents. The qualitative similarities in action and site of action (lack of selectivity) of the compounds we hope to study implies that similarities exist among the mechanisms by which the drug molecules interact with cells. Specifically, the inference seems justified, and susceptible to experimental testing, that similar parts of grossly different molecules react similarly with identical cell receptors.

2. Once chemical-structural elements common to drugs which act on nicotine-sensitive mechanisms have been identified, we hope to study quantitative differences in these common elements. We hope to explain quantitative differences in drug action and selectivity in terms of quantitative differences in the common elements. We wish to investigate the mechanism of action of nicotine by means of a comparative study of the relationship between structure and action among a number of compounds which have actions similar to one or more of the actions of nicotine.

C. Methods.

1. In vivo methods.

a. We anticipate using two in vivo methods: one method to permit the study of drug effects on transmission of nerve impulses in sympathetic ganglia, one method to permit studying the effects of drugs on nerve impulse transmission at the skeletal neuromuscular junction. Four questions are to be answered on the basis of the results of these experiments:

1. Have all of the drugs to be studied qualitatively similar, but quantitatively dissimilar, actions, or are the actions of some of the drugs qualitatively dissimilar from those of other agents?

2. Are there agents which only facilitate transmission, others which only inhibit transmission, and others which, like nicotine, may both increase and decrease function depending on the dose of the agent used?

3. To what extent are apparent differences in drug action to be referred to differences in experimental procedure (parameters of stimulation, criterion of drug effect, dose range studied, etc.) and to what extent may they be referred to actual differences in action among the drugs themselves.

4. What similarities of chemical structure can be found among agents with qualitatively similar actions on nicotine-sensitive mechanisms?

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b. Methods to be used to study the effects of drugs on transmission of impulses through sympathetic ganglia.

1. Cats will be pithed while under ether anesthesia; artificial respiration will be provided to the cat and the effects of the ether will be allowed to disappear. Arrangements will be made to permit recording the contractions of one of the nictitating membranes, and to record the arterial blood pressure of the cat. Provision will be made for intravenous administration of drugs.

2. Perfusion of the superior cervical ganglion, on the side of the nictitating membrane the contractions of which are to be recorded, will be accomplished using one of the modifications of the method of Kibjakow. Circulation to the nictitating membrane will be preserved by anastomosis of the cut proximal end of the ipsilateral external carotid artery to the cut distal end of the contralateral lingual or external carotid artery. Perfusion of the ganglion will permit the administration of drugs to the ganglion alone, or to the nictitating membrane alone, and permit the evaluation of the effects of drugs which are active at both sites.

3. The preganglionic fibers to the perfused ganglion will be prepared so that the fibers may be stimulated electrically at predetermined parameters of stimulation. The postganglionic fibers from the ganglion will be prepared so that ganglionic and postganglionic potentials may be displayed on the oscilloscope according to the method of Eccles. Arrangements will be made to stimulate postganglionic fibers electrically.

4. We shall observe, record and quantitate the changes in postganglionic action potentials and the responses of the nictitating membrane (to various rates of preganglionic stimulation) which follow the administration to the ganglion of a wide range of preselected, graded doses of the compounds to be studied. We hope thaf quantitate our data by measuring the effect of the drugs to be studied on the frequency-effect curve of the postganglionic potentials and on the contraction of the nictitating membrane as described by Rosenblueth and Cannon.

5. All precautions will be observed in order to verify the site of action of the drugs, and to ensure that the actions observed are, in fact, attributable to the drugs. Both negative and positive control drug injections will be used; positive control drugs will be drugs which are known to affect ganglionic transmission by mechanisms other than those postulated for nicotine, e.g., anesthetic agents (Exton), and morphine (Trendelenburg).

c. Methods to be used to study the effects of drugs on transmission of impulses through the skeletal neuromuscular junction.

1. The responses of the leg muscles of the fowl to indirect stimulation are useful in differentiating among drugs which influence neuromuscular transmission (a) By causing transient depolarization of the neuromuscular junction, (b) By preventing depolarization of the junction by acetylcholine released from motor nerve terminals, (c) By potentiating the effects of acetylcholine by inhibiting cholinesterase at the junctional region. The criteria for these drug effects are, respectively, (a) Alteration of the resting tension of the muscle, (b) Diminution of the amplitude of contraction of the indirectly stimulated muscle, and (c) Alteration in the myogram of single muscle twitches evoked by nerve stimulation.

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2. We propose to use this method of study, which has been described previously (Pelikan, Smith and Unna; and Theraleff and Unna): Myograms of the tibialis anterior and soleus muscles of the unanesthetized chicken are to be recorded mechanically. Contraction of the muscles is induced by electrical stimulation of the appropriate branches of the sciatic nerve. Drugs may be administered either intravenously or by retrograde injection into the ischiadic artery. Various parameters of stimulation may be used to permit the determination of frequency-effect curves.

3. The methods of analysing the data will be analogous to those used in studying the effects of the drugs on ganglionic transmission. The qualitative nature of the drug effect will be determined by inspection of the myogram. The effects of the drugs will be quantitated by determining their effects on the frequency-response curves of the muscles.

2. In vitro methods.

a. We hope to classify the drugs studied according to chemical structure and qualitative effects on nicotine-sensitive mechanisms in vivo. Such a classification should permit at least tentative identification of those structural features responsible for the qualitative action of the drugs. We hope to study in vitro quantitative differences among the drugs and relate these differences to quantitative differences in their chemical properties.

b. Ariens has emphasized that quantitative differences in apparent biological activity of compounds which act after combination with cell receptors (as nicotine is presumed to do) must be interpreted in terms of two fundamental properties of the drug which may vary independently of each other and which are dependent on the chemical structure of the drug: (1) Intrinsic biological activity, and (2) The affinity of the pharmacophoric groups of the drug for the cell receptors. We propose to study the intrinsic activity and affinity of nicotine and the compounds to be studied for the nicotine-sensitive mechanisms of the isolated frog rectus abdominis muscle.

c. Our preliminary results indicate that the nicotine-sensitive mechanism of the frog rectus abdominis muscle provides a good model-system on which to study the properties of drugs which act on ganglia and other neuromuscular junctions. The isolated frog muscle is eminently suitable for precise, quantitative studies under a wide variety of experimental conditions.

d. The molecular species (ionized, unionized) responsible for the stimulatant and blocking effects of the drugs on the frog muscle will be determined by observing the effects of alterations of the pH of the medium on the apparent potency of the drugs. Similar methods have been used to determine the molecular species responsible for the actions of barbiturates, alcohols, and local anesthetics. The intrinsic activity of the optimally effective molecular species will be determined for the agents which we study.

e. We hope to measure the affinity of the drugs for the cell receptors by determining "wash-out" times of the drugs from the tissue as a measure of affinity. Preliminary studies have shown us that the "wash-out" curves for sympathomimetic amines and nicotine follow the equation for the pseudomonomolecular reaction; it might be predicted that such a curve would describe "wash-out" rates when the rate was determined by the rate of dissociation of the drug-cell complex. Our results so far indicate that the slopes of "wash-out" curves, i.e., the apparent dissociation constants, differ from compound to compound in the case of sympathomimetic amines. "Wash-out" experiments will be performed at several temperatures in order to verify, by determining the Q10, that the wash-out process is limited in rate by a chemi-

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6. Budget Plan:

Salaries	REDACTED
Expendable Supplies	2,000
Permanent Equipment	2,000
Overhead (15% of total)	1,365
Other Misc., including travel	600
Total	REDACTED

* E.W. Pelikan
Research
Assistant
REDACTED

7. Anticipated Duration of Work:

Two years; the budget plan above applies only to the first year of research.

8. Facilities and Staff Available:

Facilities and staff of the Department of Pharmacology and Experimental Therapeutics of the Boston University School of Medicine. The staff members are competent and well equipped to aid the course of this study, especially insofar as special knowledge of anatomic and histological, biochemical and physical chemical methods might be required, and insofar as special techniques might be required to record EK and study cardiovascular phenomena. No salaries are requested for persons who might aid the work in this way.

9. Additional Requirements:

None

10. Additional Information (including relation of work to other projects and other sources of supply):

It is anticipated that the research will follow the outline described above. As the study progresses, minor modifications of experimental design and technique may be necessary; the general plan of the study and its goals will not, of course, be changed.

This research program is a direct outgrowth and extension of previous investigations conducted by the applicant. These investigations have included studies of the pharmacology of the neuromuscular junction in several species, studies of the structure-activity relationships among neuromuscular blocking agents, and studies of the actions of nicotine and sympathomimetic amines on isolated intestinal strips, the frog rectus abdominis muscle, and on the superior cervical ganglion of the cat.

Application for support of this work has not been made to any other agencies.

The applicant will spend about three-fourths of his time on these investigations.

Signature /s/ Edward W. Pelikan, M.D.
Director of Project

/s/ Reimus Rosen
Business Officer of the Institution

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R: REDACTED MATERIAL

CURRICULUM VITAE OF EDWARD W. PELIKAN

DATE AND PLACE OF BIRTH: REDACTED

DEGREES:
B.S. (University of Illinois, 1948)
M.S. (University of Illinois, Department of Pharmacology, 1950)
M.D. (University of Illinois College of Medicine, 1951)

TRAINING AND EMPLOYMENT:
Research Assistant, Department of Pharmacology, University of Illinois College of Medicine, 1948-1951.
Instructor, Department of Pharmacology, University of Illinois College of Medicine, 1951-1953.
Interne, The Presbyterian Hospital of the City of Chicago, 1951-1953.
Special Projects Officer, Office of Naval Research, U.S. Navy, 1953-1955.
Assistant Professor, Department of Pharmacology, University of Illinois College of Medicine, 1953-1955.
Assistant Professor, Department of Physiology and Pharmacology, Graduate School of Medicine, University of Pennsylvania
Associate Professor, Department of Pharmacology, Boston University School of Medicine

SOCIETY MEMBERSHIPS:
REDACTED

REDACTED

REDACTED

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TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

TIRC Grant #170R1 Report No. 2

Edward R. Pelikan, M.D.
Boston University School of Medicine

Feb. 1, 1958 - Jan. 31, 1959

A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms

Since the last report was submitted we have devoted our efforts to developing a sensitive and reliable bioassay procedure to permit measuring the acetylcholine concentration of biological and other fluids which contain nicotine, tetraethylammonium ion, hexamethonium ion and other agents which normally interfere with the assay of acetylcholine itself. The assay we have developed has been used to measure the influence of nicotine, tetraethylammonium, and hexamethonium on the acetylcholine content of incubation mixtures containing guinea pig ileal segments as generators of acetylcholine.

In our last progress report we detailed the experimental results which led us to believe that the impairment of ganglionic transmission produced by nicotine (and tetraethylammonium ions and morphine) may be the result of drug-induced impairment of release of acetylcholine from pre-synaptic nerve terminals, while the impairment of transmission produced by hexamethonium ions is probably the result of an action of the drug to prevent access of acetylcholine to post-synaptic receptors. Testing our hypothesis experimentally will ultimately necessitate measuring the acetylcholine output from ganglia in the presence and absence of impairment of transmission produced by ganglioplegic agents.

METHODS:

All determinations of acetylcholine were performed using the isolated rat ileum preparation; a total of more than eighty experiments have been performed.

After a rat of either sex of about 150 gms. weight has been killed by a blow on the head, a section of ileum is removed and washed inside and out with glucose-free Tyrode's solution. A segment of ileum about 2.5 cm. long is suspended in glucose containing Tyrode's solution in a bath of 5 ml. capacity; contractions of the ileum are recorded kymographically with an ink-writing isotonic lever which provides 6-fold magnification of the contraction. Tension on the muscle varies from 0.25 to 2.0 grams, in different experiments, but is constant during any one experiment.

The muscle bath containing the ileal segment is suspended in a water bath, the temperature of which is controlled to within 0.5° C. of 37° C. The water bath serves to preheat Tyrode's solution admitted to the muscle bath via a warming coil from a reservoir. Tyrode's solution in the muscle bath and in the reservoir is aerated with oxygen. The pH of the solution in the muscle bath is about 6.8. Some of the rat ileal strips used were stored for up to six hours at 5° C. in oxygenated Tyrode's solution.

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Agents, the effects of which are to be studied, are dissolved in glucose-free Tyrode's solution and added to the bath in 0.5 ml. quantities from a 1 ml. tuberculin syringe, or are dissolved in reservoir fluid and admitted to the muscle bath through a side-arm instead of the usual drug-free solution.

The Tyrode's solution used in the experiments has the following composition in grams/liter:

Sodium Chloride	8.0
Potassium chloride	0.2
Calcium chloride dihydrate	0.2
Sodium bicarbonate	1.0
Sodium dihydrogen phosphate	0.05
Magnesium chloride hexahydrate	0.1
Glucose	1.0

Concentrations of drugs are expressed in grams per milliliter in exponential form. Drugs are prepared as stock solutions in glucose-free Tyrode's solution; stock solutions of acetylcholine (1 mg./ml., or 10^{-3}) are acidified to pH 2 with acetic acid. Solutions are prepared for use by dilution of stock solutions with glucose-free Tyrode's solution. Fresh stock solutions are prepared every three to five days and are refrigerated at 5° C. when not in use.

RESULTS:

The more than eighty experiments performed were done in groups which differed slightly from each other in experimental design. We shall summarize the work by describing the results obtained in each type of experiment. Since the rat ileum has hardly been used in biological assays, a large number of our experiments were performed in order to characterize the biological preparation.

A. In the first series of eleven experiments, concentrations of acetylcholine between 10^{-9} and 10^{-5} were used. The effect of each concentration was studied after exposure of the tissue to that concentration for periods from 0.25 to 2 minutes. After each exposure the tissue was washed three times at one minute intervals with drug-free Tyrode's solution; drug administrations were repeated at five minute intervals. Then a dose-effect curve for acetylcholine was determined, using three to five dose levels for each curve, and from five to twelve observations in each curve. Data for two such curves were determined similarly in each experiment; between the two determinations of the curves, the muscle was exposed to an acetylcholine concentration of 10^{-4}.

For concentrations of acetylcholine between 10^{-8} and 10^{-5}, inclusive the modal duration of exposure to produce maximum contraction of the muscle was 1 minute; in all subsequent experiments the tissue was exposed to

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acetylcholine for 1 minute. For concentrations of acetylcholine between 10^{-8} and 10^{-4}, inclusive, the muscle relaxed completely, so that the tone was the same as that before drug administration, after one or two changes of bath fluid; in all subsequent experiments the tissue was washed three times at one minute intervals after each exposure to acetylcholine.

It was found that log dose-probit response curves for the system were essentially linear. In all the experiments and all subsequent experiments, responses of the muscle (measured in millimeters from the kymograph tracing) were expressed as a percentage of the maximum response of the muscle observed during the course of the experiment. The percentages were converted to probits and plotted against the logarithms of the corresponding acetylcholine concentrations; straight lines fitting the plotted data were fitted by eye.

The slopes of the two dose-effect curves determined in each experiment were computed, as were the median effective acetylcholine concentrations (EC50, determined by interpolation). The data were summarized using the non-parametric tests of Wilcoxon (Biometric Bull. 1:80, 1945) and Jackson and Ross (Am. Stat. Assoc. J. 50:416, 1955).

The mean EC50 for the curves determined before exposure to the high concentration of acetylcholine was 7.2×10^{-8} and ranged from 1.7×10^{-8} to 7×10^{-7}; the mean EC50 after exposure to the high concentration of acetylcholine was 6.1×10^{-7} and ranged from 5.6×10^{-8} to 5.4×10^{-5}. These means were statistically different ($P<0.01$). Analysis of the mean change in EC50 for the experiments indicated also that a significant ($P<0.05$) decrease in sensitivity of the muscles occurred after exposure to concentrations of acetylcholine of 10^{-4}. Observation of the effects of constant concentrations of acetylcholine (10^{-6}) repeated at intervals throughout the experiment indicated that no significant or consistent changes in responsiveness of the tissue occurred after exposure to concentrations of acetylcholine of 10^{-5} or less.

The mean slope of the dose-effect curves before exposure to the high concentration of acetylcholine was 0.782 and ranged from 0.50 to 1.20; the mean slope after exposure to the high acetylcholine concentration was 0.771 and ranged from 0.45 to 1.20. The mean slopes were insignificantly different at the 0.1 level. Paired comparison also revealed that no significant change in slope ($P>0.1$) occurred after exposure of the tissues to high concentrations of acetylcholine.

The results indicated that the rat ileum was reasonably sensitive to the spasmogenic effects of acetylcholine, and that the results from rat to rat were reasonably consistent. The system seemed appropriate to the development of a straight-line assay since even the loss of sensitivity (increased EC50) caused by high concentrations of acetylcholine was not acompanied by loss of responsiveness of the tissue and possible loss of parallelism of dose-effect curves involved in an assay.

B. In a series of four experiments an assay system for acetylcholine was designed and tested. Each experiment was composed of from eight to ten

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twenty-minute periods. During each period, the tissue was exposed to four logarithmically spaced concentrations of acetylcholine (10^{-7} to 3×10^{-6}) in random order; two of the concentrations were designated as "known," two as "unknown." The data collected during each period were used to draw two two-point log dose-probit effect curves, one for the "known" and one for the "unknown"; the EC50's and slopes of the two curves were determined. The data were summarized statistically using the methods described above and by using the Spearman rank-difference correlation coefficient.

When the mean differences between the EC50's for the "known" samples ($EC50_k$) and the "unknown" samples ($EC50_u$) were determined, it was found that in none of the four experiments was there a significant mean difference in the EC50's ($P>0.05$); similarly, in three of the four experiments there was an insignificant mean difference ($P>0.05$) in the slopes of the dose-effect curves for the "known" and "unknown" samples. In any given period the two EC50's determined were independent estimates of the same theoretical EC50 and the two slopes determined were independent estimates of the same theoretical slope. In any given period the acetylcholine content of an "unknown" sample could be measured reliably.

When the $EC50_k$'s or $EC50_u$'s, respectively, were correlated with the time during the experiment at which they were determined, it was found that the correlation coefficient was of positive sign in seven of eight cases, but that the magnitude of the coefficient was significantly different from zero ($P<0.01$) in only three of eight cases. A similar analysis of the slopes of the dose effect curves showed that slope was negatively correlated with time in all cases but that the correlation was significant in only two of eight cases. Although the sensitivity of the preparations tended to decrease with time (increase of EC50) and the reactivity tended to decrease with time (decrease in slope), the changes were usually not significant.

When, for each experiment, $EC50_u$'s were correlated with $EC50_k$'s, it was found that in three of the four cases the degree of correlation was insignificant ($P>0.05$); similarly the correlation within each experiment between the slopes of the dose-effect curves for the "known" and "unknown" samples were insignificant ($P>0.05$) in three of four cases. During any experiment, although the preparations tended to lose sensitivity and reactivity with the passage of time, the changes in $EC50_u$ and $EC50_k$ varied essentially independently of each other; the same was true of the slopes of the curves determined for the "known" and "unknown" solutions.

In none of the experiments was there a significant (P equal to or less than 0.05) correlation between $EC50_u$'s and their corresponding slopes or between $EC50_k$'s and their corresponding slopes. In the course of an actual assay one would not anticipate a bias in estimates of acetylcholine concentration of the solution as a result of changes in slope of the dose-effect curve, at least when the degree of change was of the order of magnitude observed in these experiments.

A "4-point assay" of acetylcholine would be a reliable procedure, insignificantly influenced by "aging" of the preparation. Variability in

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estimated acetylcholine concentration of a solution of unknown acetylcholine concentration was observed to occur randomly and could be minimized by replicate testing of the unknown solution.

C. A total of 32 experiments were performed to determine the effect of nicotine, hexamethonium, and neostigmine on the response of the rat ileum to acetylcholine. The experiments varied in design and in the sequences in which the various agents were administered. Because of the variability in design, uniform statistical treatment of the data was not possible, and we shall summarize the results of the experiments qualitatively.

(1) In a series of eight experiments in which the ileal strip was exposed for one minute periods alternately to varied concentrations of nicotine or acetylcholine, it was observed that nicotine in concentrations as high as 10^{-4} produced contractions of the ileum which were never more than 10% of the maximum contraction obtainable with acetylcholine. Concentrations of nicotine between 10^{-7} and 10^{-5}, inclusive, rarely produced contractions which were more than 2-3% of the maximum contraction height produced with acetylcholine. Concentrations of nicotine as high as 10^{-5} did not alter the response to subsequent administrations of acetylcholine; concentrations of nicotine of 10^{-4} decreased remarkably the sensitivity (increased the EC50) and reactivity (decreased the slope of the dose-effect curve) of the ileal strip to subsequent administrations of acetylcholine.

(2) In twenty-two experiments, dose-effect curves for acetylcholine were determined before, during, and after the rat ileal strip had been bathed in Tyrode's solution containing various concentrations of neostigmine (10^{-7} - 10^{-5}), nicotine (10^{-5}) and hexamethonium (10^{-5}), or combinations of these for periods of from twenty minutes to three hours. Concentrations of neostigmine of 10^{-7} left unchanged or decreased slightly the sensitivity of the preparation of acetylcholine; concentrations of neostigmine of 10^{-6} and 10^{-5} almost uniformly decreased the sensitivity to acetylcholine. When the sensitivity to acetylcholine was not altered by neostigmine, it was not altered by the subsequent addition of nicotine or hexamethonium to the Tyrode's solution.

D. A total of seventeen experiments have been performed using our assay system to measure acetylcholine release from the isolated guinea pig ileum, and to determine the effects of nicotine, hexamethonium and tetraethylammonium on this release.

After a guinea pig is killed, its entire ileum is removed and washed inside and out with magnesium-free Tyrode's solution. (Except for the absence of the magnesium chloride the Tyrode's solution has the composition given above.) The ileum is cut into 12-16 pieces of about equal size; a random pair of these segments are patted dry, weighed and placed into a small beaker containing 10 ml. of oxygenated magnesium-free Tyrode's solution containing neostigmine, 10^{-6}. Six such beakers are prepared. The contents of one beaker receives no further treatment. Two of the beakers are placed in boiling water for 10-20 minutes, and reconstituted to 10 ml. volume with oxygenated magnesium-free Tyrode's solution; one of these beakers

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receives no further treatment; to one is added the highest concentration of nicotine, hexamethonium, or tetraethylammonium to be studied in the experiment. To each of two other beakers is added one of two concentrations of the agent (nicotine, hexamethonium, or tetraethylammonium) to be studied. A sixth beaker, like the first, contains only neostigmine. All of the beakers are kept at 37° C. for at least one hour. In the case of all beakers but the first described above, after reconstitution or introduction of the nicotine, hexamethonium, or tetraethylammonium, or introduction of neostigmine the incubation of the beaker at 37° is carried out for five minutes; then the fluid contents of the beaker are removed, and immediately replaced by 10 ml. of fluid of identical composition, and incubated for 1 hour. Oxygen is bubbled into each beaker during incubation.

After the one hour incubation period, 6 ml. of fluid are removed, diluted with 3.0 ml. of acetic acid solution of pH 3-4, diluted further with Tyrode's solution containing neostigmine (10^{-6}) and assayed against standard concentrations of acetylcholine, using a 4-point parallel-line assay using a random dose order. Equipotent quantities of the standard and unknown solutions are equvalent to about an EC20 (concentration required to produce a contraction height 20% of the maximum obtainable). Using the data, the acetylcholine output from the guinea pig ileal segments are calculated in units of micrograms of acetylcholine/gram of tissue wet weight/hour. All samples are assayed in duplicate; the two results are averaged. Comparisons between the means are made by means of the "G" test of Jackson and Ross.

After each sample has been assayed in duplicate, the rat ileum is exposed to atropine (10^{-5} - 10^{-4}), and both standards and the largest volume of each sample tested previously are "reassayed." In no case, in which the response of the ileum to the standard has been abolished, have unknown solutions produced shortening of the ileal strip.

RESULTS:

In two preliminary experiments it was observed that the acetylcholine output in the presence of neostigmine (10^{-6}) and after an incubation of one hour, was 0.625 and 0.602 micrograms/gram/hour, respectively. Under similar conditions, but in the presence of nicotine (10^{-5}), the acetylcholine outputs were 0.502 and 0.232 mcgms/gm/hr., respectively. When after five minutes of incubation in the presence of nicotine (10^{-5}) the fluid contents of the beakers was discarded and immediately replaced before incubation for one hour, the acetylcholine outputs for the one hour period were 0.183 and 0.000 mcgm/gm/hr., respectively. We interpret these results to mean that immediately after administration of nicotine to the ileal strip, rather large amounts of acetylcholine may be released, i.e. the "stimulant" phase of nicotine action occurs; this acetylcholine accumulates in the incubation medium and, if not removed, can mask the decreased amounts of acetylcholine released during the "paralytic" phase of nicotine action. In all subsequent experiments the effects of nicotine, hexamethonium, and tetraethylammonium on acetylcholine output were measured during the one hour period following the preliminary five minute incubation period.

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In order to determine whether administration of neostigmine might have early effects on acetylcholine output, as might be anticipated from the work of Riker, et al. (J. Pharmacol. 121:280, 1957) we measured acetylcholine output from strips incubated for one hour in the presence of neostigmine alone and from strips, similarly treated with neostigmine, but incubated for one hour only after a preliminary five-minute incubation period. The data are given in the first two columns of the table. Analysis of these data indicate that the mean difference in mean acetylcholine output under the two conditions was insignificantly different from zero ($P>0.05$), i.e. neostigmine had no significant effect on acetylcholine during the first five minutes of incubation.

The results of the experiments are given in the table.

It can be seen that in the experiments using nicotine, the mean acetylcholine release in unboiled tissue treated with only neostigmine was 0.492 mgm/gm/hr. Solutions which had contained guinea pig tissue heated in a boiling water bath caused no contraction of the rat ileum. Such solutions still produced no response in the rat ileum even when they contained nicotine (10^{-5}). Nicotine (10^{-5}) completely prevented acetylcholine release in all five experiments. Nicotine (10^{-6}) completely prevented acetylcholine release in three experiments, and increasing the amount released in two. Since in these two experiments high concentrations of nicotine abolished acetylcholine release, we interpret the data as indicating that in these two strips nicotine (10^{-6}) was a "stimulating" rather than a "paralyzing" or "blocking" concentration. All of the decreases in acetylcholine output produced by nicotine, but neither of the increases, were significant at the 0.05 level of significance.

In the experiments with tetraethylammonium, the mean acetylcholine release in unboiled tissue was 0.499 mcgm/gm/hr. Slight amounts of acetylcholine were present in boiled tissue in two experiments. In every experiment tetraethylammonium ion (10^{-6} to 10^{-3}, inclusive) increased the amount of acetylcholine present in the incubation fluid, occasionally by several hundred per cent, but the increase was significant ($P<0.05$) in only three of ten cases. There is no clear relationship between tetraethylammonium concentration and amount or increase in acetylcholine concentration.

The mean acetylcholine release in experiments with hexamethonium was found to be 2.126 mcgm/gm/hr. for the unboiled tissue. The incubation fluid for boiled tissue was found to contain no acetylcholine; in one of three instances fluid for boiled tissue with added hexamethonium (10^{-4}) was found to contain acetylcholine. In all three experiments with hexamethonium (10^{-6} to 10^{-3} inclusive) the inclusion of hexamethonium in the incubation fluid resulted in a decreased measurable amount of acetylcholine, but the difference was significant ($P<0.05$) in only one of six cases. In two of three experiments there was less acetylcholine assayed in the incubation fluid containing the higher concentration of hexamethonium.

When the effectiveness of standard concentrations of acetylcholine were correlated with the time during each assay at which the respective concentrations were administered, it was found, in the case of experiments with nicotine

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and hexamethonium, that there was, in general, a gradual but statistically insignificant ($P>0.1$) loss in sensitivity of the rat ileum preparation, i.e. decreased response to a standard concentration of acetylcholine. In experiments with tetraethylammonium, however, there was a progressive and significant ($P<0.05$) increase in effectiveness of standard concentrations of acetylcholine during the time course of the assays.

DISCUSSION:

We have previously put forward the hypothesis that impaired ganglionic transmission produced in the cat by administration of nicotine or tetraethylammonium is the result only of a decrease in acetylcholine release from pre-synaptic nerve terminals produced by the agents. On the other hand, our previous data were consistent with the hypothesis that hexamethonium impaired ganglionic transmission by preventing access of acetylcholine (released in normal amounts) to post-synaptic cell receptors.

The results reported above, obtained from experiments with nicotine, are consistent with our hypothesis: The amount of acetylcholine determined to be present in medium in which guinea pig ileal segments had been incubated was found to be decreased by nicotine. This was particularly true when care was taken to eliminate from the incubation fluid the acetylcholine released during the early "stimulant" phase of nicotine's action.

The results obtained with tetraethylammonium in these experiments are difficult to interpret, because the tetraethylammonium ion is known to have a number of actions which, exerted independently, but simultaneously, could yield the results we have observed. Under our conditions of incubation, and in the absence of detailed knowledge of processes in the guinea pig ileal wall, it is impossible to decipher the meaning of our results.

Tetraethylammonium ion, for example, has been shown to facilitate benzoylcholine hydrolysis by serum cholinesterase (Erdos, E., et al. Science 128:92, 1958); such an effect in our preparation would tend to decrease the amount of acetylcholine present in the incubation fluid. Kensler and Elsner, however, have shown that in the presence of low acetylcholine concentrations, tetraethylammonium decreases specific cholinesterase activity, but increases activity in the presence of high acetylcholine concentrations (J. Pharmacol. 102:196, 1951). This effect of tetraethylammonium was not observed by Kensler and Elsner when serum cholinesterase was used. Since we have no quantitative information on the kinds, concentrations, and kinetics of cholinesterases in the guinea pig ileum, little information of substrate concentration at the esteratic sites, and no information on the interaction of tetraethylammonium and neostigmine in the system, our results cannot easily be interpreted in terms of the effect of tetraethylammonium on the stability of acetylcholine after its release from nerve terminals. Tetraethylammonium might have other actions in the preparation which could account for our results. Stovner (Acta Pharmacol. et Toxicol. 15:55, 1958) and Koketsu (Am. J. Physiol. 193:213, 1958) have both presented evidence that tetraethylammonium can cause acetylcholine release at the skeletal neuromuscular

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junction, and Cowan, et al. (J. Physiol. 91:101, 1937) have shown that tetraethylammonium can increase the spontaneous firing of at least isolated neurons. Such mechanisms as these could account for the results we have observed, could it be shown that the mechanisms were operative in the guinea pig ileum.

Paton and Zaimis (Brit. J. Pharmacol. 6:155, 1951) have reported that hexamethonium did not decrease acetylcholine output from the cat superior cervical ganglion at the time transmission was impaired by hexamethonium. However, Kensler and Elsner (ibid.) have reported that like tetraethylammonium, hexamethonium can increase or decrease specific cholinesterase activity, depending on acetylcholine concentration. We lack sufficient quantitative information to determine whether the phenomenon observed by Kensler and Elsner could account for our observation that less acetylcholine was present in incubation medium which contained hexamethonium than in medium which did not.

It should be pointed out that our assay procedure measures the acetylcholine content of incubation fluid; we can only infer that, in the case of nicotine, for example, total absence of acetylcholine in medium which contained low concentrations of nicotine is more likely to indicate impaired release of acetylcholine than only facilitations of its hydrolysis. Although the guinea pig ileum has proven to be a useful "acetylcholine generator" in the development of our assay procedure, it is not the present purpose of our work to study in detail the effects of agents on the guinea pig ileum, which contains several systems for release and degradation of acetylcholine. Hence, future work will follow two lines of endeavor:

(1) Assay of acetylcholine output from the cat superior-cervical ganglion. Our hypotheses of the mechanism by which nicotine, tetraethylammonium and hexamethonium impaired ganglionic transmission were derived from experiments using the relatively simple ganglionic transmission system. We shall use our assay procedure to measure acetylcholine content of the venous effluent from ganglia in which cholinesterase has been inactivated, during periods of normal transmission and when transmission has been impaired by the administration of nicotine, tetraethylammonium, and hexamethonium.

(2) The results obtained with tetraethylammonium and hexamethonium, particularly the change in sensitivity of the assay preparation after repeated exposure to tetraethylammonium, suggests that further investigations be made to determine the dependency of our results on the concentrations of tetraethylammonium and hexamethonium and the degree to which various concentrations of the agents influence the responsiveness of the assay strip.

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Effects of Nicotine, Tetraethylammonium and Hexamethonium on Apparent Acetylcholine Output (in mcgms/gm/hr) from Isolated Guinea Pig Ileal Segments

Experiment	Drug	Conditions of Incubation*							
		Unboiled Tissue +	Unboiled Tissue	Boiled Tissue	Boiled Tissue Plus Drug	Unboiled Tissue Plus Drug			
						Drug 10^{-3}	Drug 10^{-4}	Drug 10^{-5}	Drug 10^{-6}
69	Nicotine	0.327	0.346	0.0	0.0	-	-	0.0 ‡	0.401
70		1.477	0.494	0.0	0.0	-	-	0.0 ‡	0.0 ‡
71		0.437	0.695	0.0	0.0	-	-	0.0 ‡	0.0 ‡
72		1.165	0.432	0.0	0.0	-	-	0.0 ‡	0.557
73		1.026	0.495	0.0	0.0	-	-	0.0 ‡	0.0 ‡
	Mean	0.887	0.492	0.0	0.0			0.0	0.194
77	Tetra-ethyl ammonium	1.105	0.342	0.0	0.0	-	0.612	0.575	-
78		0.554	0.458	0.0	0.0	-	0.792‡	0.844‡	-
79		0.207	0.257	0.018	0.0	-	0.657	0.268	-
80		2.062	0.710	0.0	0.0	1.589‡	1.063	-	-
81		0.864	0.726	0.0	0.747	-	-	0.887	1.261
	Mean	0.958	0.499	0.003	0.149	1.589	0.881	0.643	1.261
82	Hexa-methonium	0.740	3.081	0.0	0.0	-	-	1.055	0.614
83		1.138	0.715	0.0	0.314	-	0.0‡	0.610	-
84		0.890	2.583	0.0	0.0	0.915	1.207	-	-
	Mean	0.923	2.126	0.0	0.105	0.915	0.604	0.833	0.614

* All incubations were performed in the presence of neostigmine, 10^{-6}.

\+ Incubations performed for one hour; for other columns, incubations performed for one hour after preliminary five minute incubation (see text).

‡ Difference from corresponding control value significant at 0.05 level of significance.

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

Extremely broad proposal
Have all of these & unknown components been worked out by experts

Application For Research Grant

Date: November 24, 1954

1. Name of Investigator: Frank D. Pickel, Ph.D.

2. Title: Associate Director of Research

3. Institution & Address: Evans Research & Development Corp.
250 East 43rd Street
New York 17, New York

4. Project or Subject: Chemical Studies of Tobacco, Tobacco Additives, and Cigarette Smoke

a. Isolation and identification of products of controlled combustion of tobacco and of tobacco and of cigarette tobaccos containing the usual additives.

b. Acting as a central source of supply, for medical investigators, of compounds identified in cigarette smoke; preparation by isolation or synthesis.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Smoking is a controlled combustion that initiates a variety of chemical reactions covering the extreme range from no reaction, as shown by the survival of unchanged nicotine, to complete destruction, as shown by the presence of carbon monoxide and carbon dioxide in the main smoke stream. It is a process that simultaneously includes such unit operations as evaporation, sublimation, distillation, steam distillation, and pyrolytic cracking.

Because this process is complex and because the modern cigarette has become complicated by the incorporation of additives (casing materials, humectants, etc.), it is suggested that representative tobacco samples wrapped in pure alpha cellulose be studied first to obtain a basic set of values with which the values obtained by the study of more complex formulations may be compared.

why how

This approach to the problem is dependent upon the machine used to perform the necessary controlled smoking operation to collect representative samples for analysis. Evans Research and Development Corporation has a machine that will smoke several cigarettes simultaneously under controlled and reproducible conditions. Moreover, the performance of the machine is recorded continuously. It is proposed to construct a large machine based on the principles and design of the present one. This machine, therefore, will insure a constnat supply of representative analytical samples.

research

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5 continued -

The separation of cigarette tobacco smoke into its condensible and non-condensible fractions and the separation of the condensible fraction into its acid, basic, and neutral fractions would be the first step to be undertaken. The individual components of these fractions then would be separated and identified by chemical and physical methods such as spectrophotometry, colorimetry, chromatography, molecular distillation, distribution between immiscible solvents, derivatization, etc.

Sadler Job Note # req'd

In addition to the analytical work on tobacco smoke, and smoke from cigarettes containing known additives, research would also be directed toward the isolation and identification of the components of the tobacco itself. Examination of the data from both studies should give a clue to the chemical and physical phenomena involved in cigarette smoking.

Such as

Wow!

The analytical studies could be supplemented by a program involving the preparation of the known components of tobacco smoke either by isolation or synthesis. The availability of these pure materials would be helpful in working out suitable quantitative analytical procedures. They might also be useful in other laboratories that are studying tissue changes under various conditions.

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6. Budget Plan:

Salaries	$15,750
Expendable Supplies	2,000
Permanent Equipment	2,000
Overhead	9,375
Other (travel)	500
Total	$30,125

30 or 300

7. Anticipated Duration of Work: The budget indicated above is for one year. It is anticipated that a total of three years will be required. An annual budget review is suggested in order to provide for changes in accordance with new developments.

8. Facilities and Staff Available:

Consultant: Dr. Gilbert Stork, Columbia University

Regular Staff: Specialists from among a staff of 40 chemists will be selected and assigned to those portions of the investigation for which they are highly trained. These chemists will be made available to the research program in accordance with the requirements as determined by Dr. Frank D. Pickel, the principal investigator.

Facilities: Well equipped chemical and physical laboratories, including machine shop and other supplemental services, with 17,500 square feet in New York City, and 5,000 square feet in Yonkers, New York.

9. Additional Requirements:

The principal addition to present facilities will be the construction, in the Evans Research and Development Corporation machine shop, of a larger model of the cigarette smoking machine now in use.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Over a period of years the laboratories of Evans Research and Development Corporation have been engaged in several projects concerned with evaluating filters both for cigarette holders and for cigarette tips. They have also been actively engaged in non-conventional chemical analysis; the separation and identification of trace components from such diverse material as pepper, maple syrup, dehydrated foods, hair, prophyrins, natural gums and resins, contaminated air, alcoholic beverages, paper and foods. In the pharmaceutical field, projects dealing with antibiotics, radio opaque materials, hormones, carcinogenic hydrocarbons, enzymes, vesicants, and diuretics have been successfully carried out. Its staff has had many years experience in such fields and is familiar with the techniques which facilitate such work.

The success obtained with nonconventional chemical analysis might be of immediate interest to the Tobacco Industry Research Committee. The term, nonconventional chemical analysis is used here to distinguish it from orginary routine analytical procedures. This kind of analysis should be useful in studying the chemical composition of tobacco and accompanying products.

Signature /s/ Eric J. Hewitt

Director of Project Eric J. Hewitt, Ph.D.
Vice President and Director of Research

/s/ James Lawrie

Business Officer of the Institution
James Lawrie, Secretary-Treasurer

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The investigation of black pepper, undertaken by Evans Research and Development Corporation as a research project for the Office of the Quartermaster General of the United States Army, can be cited as an example of the successful use of nonconventional analysis. Natural pepper was analyzed qualitatively and semi-quantitatively. Domestic materials containing the components of natural pepper were then formulated in such a way as to duplicate the odor and flavor of natural pepper. A synthetic bit principle and a substitute oleorasin (as a fixative) were added to this formulation. The result was a highly satisfactory "synthetic" pepper which is now being field tested by the Army.

Evans Research and Development Corporation is well qualified to study the combustion phases of the cigarette problem because of their extensive experience with solid fuels and explosives.

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Committee:
Little Chm.
Reimann
Lynch

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TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street
New York 17, N. Y.

Application for Research Grant

Date: September 1, 1959

1. Name of Investigator: Otakar J. Pollak, M.D., Ph.D.

2. Title: Executive Director

3. Institution & Address: Dover Medical Research Center, Inc., 9 Kings Highway, Dover, Delaware

4. Project or Subject: Effect of tobacco derivatives on arterial and myocardial tissue cultures.

5. Detailed Plan of Procedure:

To this date, but a single publication on human aortic and myocardial tissue cultures is available and but a single publication on cholesterol uptake by human aortic cultures. No references were found by us concerning tissue cultures of healthy or diseased heart or blood vessels of other species. No mention of tobacco derivatives with reference to cardiovascular tissue cultures was found by us in the literature.

Presently, we are engaged in culturing normal and diseased cardiovascular tissues from rabbits. These studies are to be expanded to tissues from other species, including human material obtained surgically.

We propose to study the influence of available tobacco derivatives -- in varying dose and in combinations -- upon the speed, quantity, and quality of cellular growth in cardiovascular tissue cultures. Continuation of the project trying to counteract adverse effects may be warranted. Obviously, this has to await results of the proposed study.

Cultures are to be observed under the microscope and phase microscope and the growth to be recorded by photography. Warburg cell metabolism studies are to be used supplementary to microscopic methods.

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6. Budget Plan:

Salaries - Ph.D.	REDACTED
Expendable Supplies - Media, glassware	2,400.--
Permanent Equipment - 0	0.--
Overhead - 10%	960.--
Other - Films, processing	600.--
Total	REDACTED

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7. Anticipated Duration of Work: 1 year (Continuation to depend upon results of first project. See last paragraph under 5, above.)

8. Facilities and Staff Available: The Dover Medical Research Center, Inc., is equipped for tissue cultures (incubators, centrifuges, homogenizer, water baths, microscope, phase microscope, microphotography, etc.), for study of cell metabolism (Warburg), for biochemistry, electrophoresis, chromatography, etc., (Spectrophotometer, pH meter, balances, chromatograph, electrophoresis, etc.)

O. J. Pollak, M.D., Ph.D. (author of over 130 publications -- half of which deal with atherosclerosis and related problems; pathologist) -- to direct all projects.

Hassan Rouhandeh, Ph.D. - engaged in tissue cultures and cell metabolism (started on June 1, 1959).

Tatsuo Kokubu, M.D., Ph.D. - engaged in tissue cultures in Osaka, Japan, (to start as Exchange-Visitor, January 1, 1960).

Araceli F. de Engel, Ph.D. - engaged in electrophoresis, chromatography, biochemistry of blood and blood vessel extracts (started June 1, 1959).

Donald L. Howie, M.D., (Associate Pathologist to Dr. Pollak;) - to start own project on January 1, 1960.

9. Additional Requirements: Will need aid in obtaining full line of tobacco derivatives.

10. Additional Information: The Dover Medical Research Center, Inc., is a non-profit organization incorporated in the State of Delaware, exempt from taxation and eligible to accept tax-free grants by the Department of Internal Revenue, approved for student-exchange visitors by the State Department.

At present, the CENTER is operating under research grants from the National Heart Institute (N.I.H.), the Delaware Heart Association, and a Pharmaceutic Firm. Applications for additional grants in aid of research have been made to the American Heart Association, Life Insurance Research Fund, and the National Vitamin Foundation. -- None of the current grants or of the applications are for the project proposed to the Tobacco Industry Research Committee.

Additional information will be forwarded upon request. Project site visitation or interview would be welcome.

Signature O. J. Pollak

Director of Project - O. J. POLLAK, M.D., Ph.D.

Thomas J. Deacon

Business Officer of the Institution- Thomas Deacon

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CURRICULA VITAE OF RESEARCH STAFF OF DOVER MEDICAL RESEARCH CENTER, INC.

A. Otakar J. Pollak, Principal investigator:
Masaryk University, School of Medicine, Brno, 1924-1930; M.D. 1-15-1930
Ph.D. 6-10-1934 (Chem.)

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Hospitals: St. Anna County Hosp., Brno, 1939-38; Taunton State Hosp., Taunton, Mass., 1940-44; Wilmington Gen. Hosp., Wilmington Del., 1945-48; Quincy City Hosp., Quincy, Mass., 1948-51; Kent Gen. Hosp., Dover, Del., Milford Mem. Hosp., Milford, Del., Beebe Hosp., Lewes, Del., 1952-on; Consultant, U.S. Air Base Hosp., Dover, Del., Welfare Home & Hosp. for Aged, Smyrna, Del., Hosp. for Mentally Retarded, Stockley, Del, 1952-on or 58-on. - Pathologist.
Academic: Ass't. Prof. Path., Masaryk Univ., Brno, 1931-38; Research Assoc. Dept. Legal Med., Harvard Med. School, Boston, Mass., 1939-40; Special Lecturer, Pathology, Womans' Med. Coll., Philadelphia, Pa., 1944-48; Ass't. Prof. Pathology, Hahnemann Med. College, Philadelphia, Pa., 1954-on, Exec. Director, Dover Medical Research Center, Inc., 1959 and on.
137 publications including a book, excl. panels, etc.

B. Hassan Rouhandeh, Collaborating investigator:
Kansas State University, Manhattan, Kansas, 1951-1959; B.S. 1955.
M.S. 1957, Ph.D., 1959.

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Graduate Research Assistant, Dept. Bacteriology, Kansas State University. Manhattan, Kansas, 1956-59; Research Associate, Dover Med. Research Center, Dover, Del., 1959-on.
4 publications.

C. Tatsuo Kokubu, Collaborating investigator:
Osaka University, Medical School, Osaka, Japan, 1945-51; M.D., 1951.
Osaka University, School of Philosophy, Osaka, Japan, 1953-58; Ph.D., 1958.
Osaka University Hospital, Osaka, Japan; interne 1951, resident, 1952-1958; Assistant in Internal Medicine (Prof. I. Donomae), 1958-59; Research Assoc., Dover Medical Research Center, Dover, Del., 1960-on.
13 publications

D. Araceli Magda Fortea DeEngel, Collaborating investigator:
University of Litoral, Rosario, 1943-47; B.S. in Pharmacognosy, 1947; 1947-49, B.S. in Biochemistry, 1949; 1950-54, Ph.D., in Pharmacology and Biochemistry, 1954.
Associacion Bioquimica Argentina, 1947, Associacion Quimica Argentina, 1947, Sociedad Biophysica Argentina, 1947.
Ass't. Prof. of Applied Physics, 1944-45, of Pharmacognosy, 1947-49, of Biochemistry, 1949-50, University of Litoral, Faculty of Medical Science, Rosario; Technical Ass't. Bromatological Institute, Province of Santa Fe, 1949-56; Technical Director, Laboratory Marxer, Buenos Aires, 1957-59; Research Associate, Dover Medicall Research Center, Inc., 1959 and on.
7 publications and translation of book.

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E. Donald L. Howie, M.D.
Washington University School of Medicine, 1942-46; M.D., June 1946.

REDACTED

Colorado State Hosp., Pueblo, Col., 1950-51; Kern Gen. Hosp., Bakersfield, Calif., 1951-52 & 1954-56; Navy, 1952-54; Fairfield State Hosp., Newtown, Conn., 1956-59, Dir. of Labs., Milford Mem. Hosp., Milford, Del. Beebe Hosp., Lewes, Del., Kent Gen. Hosp., Dover, Del., Oct. 1959-on, Assoc. Pathologist 2 publications

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Bioassay, carcinogenesis and tissue culture #252R1

Committee:
Dr. Little, Chairman
Dr. Reimann
Dr. Lynch

#252 activated 4/1/60

Solomon

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty-second Street New York, N.Y.

Application for Research Grant

November 10, 1960

1. Name of Investigator: Otakar J. Pollak, M.D., Ph.D.

2. Title: Executive Director

3. Institution & Address: Dover Medical Research Center, Inc.
9 Kings Highway
Dover, Delaware

4. Project or Subject: Effect of Tobacco Derivatives on Arterial and Myocardial Tissue Cultures.

5. Detailed Plan of Procedure:

The plan of procedure as outlined in the original application need not be changed. As evident from the attached progress report for the first six months of the current study actual experimentation with nicotin had to be delayed pending the establishing of base lines, i.e., the finding of optimal working conditions for rabbit tissue. Similarly, work with human tissues has to be posponed until the establishing of ground rules based on results of study of rabbit tissues.

6. Budget Plan:

Salaries	$ 7,600.00
Expendable Supplies	2,400.00
Permanent Equipment	----
Overhead (15%)	1,000.00
Other	600.00
Total	$11,600.00

7. Anticipated Duration of Work:

At least, a second year (April 1, 1961 - March 31, 1962). Results may warrant further extension.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

8. Facilities and Staff Available:

The Dover Medical Research Center, Inc., is equipped for tissue cultures (incubators, centrifuges, homogenizer, water baths, microscope, phase microscope, microphotography, etc), for study of cell metabolism (Warburg), for biochemistry, electrophoresis, chromatography, etc., (Spectrophotometer, pH meter, balances, chromatograph, electrophoresis, etc.).

O. J. Pollak, M.D., Ph.D. (author of over 130 publications--half of which deals with atherosclerosis and related problems; pathologist) -- to direct all projects.

Tatsuo Kokubu, M.D., Ph.D. - engaged in tissue cultures in Osaka, Japan, and has been working here since February 1, 1960.

Araceli F. Engel, Ph.D. - engaged in electrophoresis, chromatography, biochemistry of blood and blood vessel extracts (started June 1, 1959). Also does Warburg studies.

Reizo Koide, M.D., of Osaka, author of several papers on atherosclerosis, will take the place of Dr. Kokubu but will arrive for a training period of three months before Dr. Kokubu's tenure expires.

9. Additional Requirements:

None

10. Additional Information:

The Dover Medical Research Center, Inc., is a non-profit organization incorporated in the State of Delaware, exempt from taxation and eligible to accept tax-free grants by the Department of Internal Revenue, approved for student-exchange visitors by the State Department.

At present, the CENTER is operating under research grants from the National Heart Institute (N.H.I.), the Delaware Heart Association, and a pharmaceutic firm, in addition to the grant from the Tobacco Industry Research Committee.

/s/ O. J. Pollak, M.D., Ph.D.
Director of Project

/s/ Thomas J. Deacon
Business Officer of the Institute

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Source: https://www.industrydocuments.ucsf.edu/docs/fhm0000

TIRC Grant #252

Progress Report #1

O. J. Pollak, M.D., Ph.D.

April 1960--September 1960

CONFIDENTIAL

DOVER MEDICAL RESEARCH CENTER, INC.
Office: 9 Kings Highway, E., P.O. Box 228
Dover, Delaware

October 3, 1960

Robert C. Hockett
Assoc. Scientific Director
TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East 42nd Street
New York 17, N.Y.

Dear Mr. Hockett:

Time is surely flying and six months have passed since the project under the grant of the Tobacco Industry Research Committee was activated.

Enclosed find a brief report which will bring you up to date. It seems that the beginning is the hardest and it is surely hoped that in the future progress will be speedier. I personally feel that our observations are very interesting and certainly warrant continuation. They also will warrant expansion utilizing material from other species...especially human material. Projecting into the future I would say that it would take us another six months to study blood vessels and heart muscle of rabbits. Many changes had to be made in the course of studies. The amount of cells does not lend itself to metabolic studies and, therefore, we have started to utilize homogenates of vascular tissue for Warburg studies. This work has, unfortunately, not yet progressed very far. We are still trying to get the base lines; that is, differences between the oxygen consumption of tissue from normal rabbits, rabbits with induced blood chemical changes but without visible vascular changes and, finally, tissues from rabbits with hypercholesteremia and hyperlipemia and, also, aortic atherosclerosis. Only after we get the base lines can we start adding nicotine in varying concentrations to these 3 types of material.

Certainly, the studies should not be confined to nicotine but will have to embrace other ingredients of tobacco as well as tobacco smoke. With this in mind, I would somehow like to imply that another application for continued support of these studies will be forthcoming. Naturally, we don't want to let the grant expire before we apply for continuation and I am awaiting your personal advice as to the proper procedure for the future.

Sincerely yours,

/s/ O. J. Pollak, M.D., Ph.D.
Executive Director

OJP:pkg

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Stanford University Libraries https://exhibits.stanford.edu/docs/tvvm0000

Effect of Tobacco Derivatives on Arterial and Myocardial Tissue Cultures

Considerable time had been spent establishing "controls", i.e.; cultures of material of healthy rabbits and from rabbits rendered hypercholesteremic. At first, spindle cells of fibroblastic character were cultured, only. It became necessary to find optimum conditions for growth. (A report on these studies has been submitted for publication.) Modifications of technics led to successful cultures of intimal endothelium cells from normal aortae and from experimental intimal plaques. (A report on these studies is being prepared for publication.)

Nicotin has been added tc culture medium in three concentrations, 1:40,000, 1:10,000 and 1:2,500. The amount of nicotin solution added to medium was 0.04 ml. per 100 ml. The two weaker solutions (1:40,000 and 1:10,000) had no effect on the number of positive cultures. In all three concentrations nicotin delayed the cell growth. In cultures from healthy aortic tissues, the number of cells was reduced by almost one-half in medium with dilute nicotin solutions (1:40,000 and 1:10,000) and by two-thirds in concentrate solution (1:2,500). Cells from atheromatous plaques were more sensitive: the number of cells was reduced by about one-third in medium with 1:40,000 nicotin solution, by almost one-half with 1:10,000 solution and by about 90 per cent in medium with 1:2,500 solution of nicotin, at a given date. Morphologic changes of fibroblasts were seen in medium with 1:2,500 solution but not with more diluted nicotin. Endothelium cells were more sensitive: they became severly vacuolized where nicotin solutions of 1:2,500 or 1:10,000 had been added to the media. Degranulation of the cells and nuclear pyknosis occurred even where 1:40,000 nicotin solution had been added. Phagocytic ability of cells was also affected. The life span of endothelium cells was considerably shortened by addition of 1:2,500 nicotin solution to the medium.

The number of animals studied is, as yet, small. The studies have to be expanded to add validity to our observations. Studies of myocardial cells are not conclusive, so far.

Amino acid assays of culture medium before planting and after cell growth have been started. The number of assays is too small to allow evaluation. Lack of "pure" cultures of endothelium cells presents a problem.

Until now, nicotin has been added to medium before planting. Addition of nicotin solutions to positive cell cultures will be started, shortly.

Since the total number of cells grown is hardly sufficient for Warburg studies and since it is hardly possible to obtain pure lines of endothelium cells it will be necessary to modify part of the research project. We plan to prepare homogenates from aorta and myocardium of healthy and of hypercholesteremic rabbits, subject these to metabolic studies and then investigate the influence of nicotin additives.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#134 B R2
Activated 11/21/56
Renewed 3/1/58

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

Application For Research Grant

RENEWAL

Date: December 2, 1958

1. Name of Investigator: C. M. Pomerat

2. Title: Professor of Cytology, Department of Anatomy, Director, Tissue Culture Laboratory

3. Institution & Address: The University of Texas - Medical Branch Galveston, Texas

4. Project or Subject: Tissue culture studies of human lung.

5. Detailed Plan of Procedure:

Using both experimental animals and human cells, we would like to continue studying the effect of various noxious chemicals on chromosomal patterns. Data is urgently needed on the toxicity of benzpyrene and related chemicals. We would like to check the suggestion that components of tobacco tars may act as co-carcinogens. No evidence for this view has been found in preliminary experiments combining Neal's fraction number 3 and benzpyrene, but the work should be extended.

Animals should be used to determine whether cells with experimentally produced dicentric chromosomes are capable of inducing neoplasms.

6. Budget Plan:

Salaries	$ 9,500*
Expendable Supplies	189
Permanent Equipment	-
Overhead (10%)	1,091
Other - Travel	1,220
Total	$12,000
Supplement to 1958 Grant (Attached sheet) #134B R1	1,500
Total	$13,500

* Includes an allowance for WCI at 3/4% and for OASI at 2¼%.

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7. Anticipated Duration of Work: One year

8. Facilities and Staff Available:

Please see previous application for facilities available.

Staff Y. H. Nakanishi (until March 30, 1959)
M. Mizutani
Y. Ohnuki (beginning April 1, 1959)

9. Additional Requirements:

Travel expenses for new worker. Round trip air ticket (trans-Pacific) and U. S. Train travel in advance at approximately $1,200.00.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Considerable interest is being shown in this work by visiting pathologists and by students of air pollution. It is possible that the National Institutes of Health might be willing to supplement our effort for studies on contaminants in the atmosphere.

/s./ C. M. Pomerat
Director of Project

/s./ E. D. Walker
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

SUPPLEMENTARY GRANT FOR 1958

Statement of Expected Expenses and Funds Available from 1958 Grant

Dr. Nakanishi - Stipend #424 - November through February 1959	$1,696.00	
Dr. Mizutani - Stipend #375 - November through February 1959	1,500.00	
Dr. Nakanishi - Travel to Japan	600.00	
Dr. Nakanishi - Stipend for March 1959	424.00	
Dr. Mizutani - Stipend #375 - April, May, June	1,125.00	
Dr. Mizutani - Travel to Atlantic City April 13-18, 1959	350.00	
	$5,695.00	
Balance on hand as of November 1, 1958	-4,500.00	(approx.)
	$1,195.00	

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

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APR 22 1973
PHILIP MORRIS, INC.
RESEARCH & DEVELOPMENT
DEPT.

https://www.industrydocuments.ucsf.edu/docs/tfvm0000

COPY

THE UNIVERSITY OF TEXAS - MEDICAL BRANCH
Galveston

CONFIDENTIAL

October 22, 1958

Dr. Robert C. Hockett
Associate Scientific Director
Tobacco Industry Research Committee
150 East 42nd Street
New York 17, New York

Dear Dr. Hockett:

Many thanks for your very comprehensive letter of October 16.

With regard to the material which we obtained from the Ecusta Paper Division, we found that it was so toxic that we could only use very dilute concentrations. Removal of the acid-base fraction would still require a dose which we feel might be too high for cell studies. We fear that the base-free method does not remove many of the non-carcinogenic constituents. I suspect that we would have to employ dilutions roughly four times greater than were needed for Fraction 3. Also, its solubility might be relatively low in acetone. On the basis of what we have done, we think that Fraction 3 of Jack Neal's was more completely rid of the non-important substance, yet retained a fluorescent compound which produced regular cytopathological effects. Does the Ecusta group have a comparable fraction of high potency? What we got from them had to be kept frozen. We feel that the effective agent for cell study should not be thermolabile at body temperature. The compound obtained from them cost $28.20 for two ampules and we had to accept the stipulation "that in any public reference to your work, no mention be made of Ecusta or Olin Mathieson Chemical Corporation as the source of the condensate unless you first obtain clearance from us." On general grounds, I would think that scientists would be far happier if investigations in this field involved raw materials and experiments entirely governed by non-commercial interests. The strength of our present work in part is that it was produced entirely by people in the academic setting, who have nothing to gain or lose as a result of whatever conclusions are reached so long as all materials are available to other workers and the methods employed are fully described. I hope you will communicate with me again on these several points before I discuss the matter of Jack Neal's reapplication to your Committee.

Doctors Nakanishi and Mizutani are in the course of studying the effect of benzpyrine alone and in combination with Fraction 3 on the chromosomal configuration of their test cells. We hope to be able to give you a provisional report before your next board meeting in early December.

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On the basis of academic commitments, Doctor Nakanishi should return to Japan in April. However, he would like to attend the Federation meetings before leaving the United States, since he will have an important paper for presentation at that time. He would like to return to the U.S.A. at a later date. In view of the fact that students trained by Professor Makino at Hokkaido University have been so very successful in this laboratory and because there is so much more work ahead, I would like to replace Doctor Nakanishi with Doctor Y. Ohnuki who is very highly recommended. Now that Doctor Mizutani is well oriented in the area of research, he could assist us in the training of Doctor Ohnuki. We would like to be able to offer him the same financial arrangements as we had for Doctor Mizutani.

Doctors Nakanishi and Mizutani were invited by Doctor J. J. Biesele to discuss their results at a graduate seminar in cytology at the University of Texas in Austin. Among those present were Doctors Oliver, Stone and T. S. Painter. In a recent visit to Austin, Doctor Painter expressed his admiration of the work these Japanese scholars are doing. I also have had evidence from the other men that they were greatly impressed with the manner in which our chromosomal studies are being attacked.

Recently, we have had several very distinguished investigators who have had an opportunity to look over the data which has accumulated under the sponsorship of the Tobacco Industry Research Committee. Among these have been Doctor John A. Milne of the Pathology Department of Glasgow University, Professor Kenneth M. Smith of Cambridge University, England, Doctor Robert Leader of the Veterinary Pathology Department at Washington State University, Pullman, Washington, and Doctor John B. Thomison of the Department of Pathology at Vanderbilt Medical School. All agree quite spontaneously that the demonstration of chromosomal changes in tissue culture as they are being studied by Doctors Nakanishi and Mizutani constitute a very basic contribution to experimental biology and medicine. We certainly hope that we can continue with this distinguished effort and appreciate the trust and interest that you have shown in the undertaking.

With very cordial regards, I am

Sincerely yours,

/s/ C. M. Pomerat
Professor of Cytology

CMP:jf

cc: Dr. Y. H. Nakanishi

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https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE #134RR1
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y.

Activated Mar. 1, 1957.
Funds paid Mar. 21, 1957
to cover Travel etc.

RENEWAL
Application For Research Grant

Date: November 22, 1957

1. Name of Investigator: C. M. Pomerat, Ph.D.

2. Title: Professor of Cytology, Department of Anatomy
Director, Tissue Culture Laboratory

3. Institution & Address: The University of Texas - Medical Branch
Galveston, Texas

4. Project or Subject: Tissue culture studies of human lung.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

a. We have been successful in cultivating human fetal lung and lung tissue from experimental animals.

b. Cultures of pulmonary tissue are being subjected to chronic treatment with low concentrations of tobacco condensates. Some inroads are being made in similar studies in which smog and its constituents are used (ozone and unsaturated hydrocarbons as suggested by the work of Haagen-Smit of the California Institute of Technology).

c. As end points regarding the effect of test chemicals on the cells, chromosome counts and records of injury to mitochondria are being made in addition to observations on growth patterns, "strain" induction and the ultimate survival of the cultures.

d. While much information has been accumulated since Dr. Yuh Nakanishi became attached to the project in March 1957, manuscripts have not been prepared since the collection of data for statistically reliable reports is still in progress.

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Powered by Tobacco Documents Online http://tobaccodocuments.org/ucsf/tivm0000

6. Budget Plan:

Salaries - Yuh H. Nakanishi	$ 5,132*
Expendable Supplies	1,000
Permanent Equipment	- -
Overhead	638
Other Travel to meetings	250
Total	7,020
For new assistant	5000
Total	12,020 00

* OASI $94.50 - WCI $37.50

7. Anticipated Duration of Work: One year.

8. Facilities and Staff Available:

The tissue culture laboratory has facilities for 1200 roller tube cultures and equipment for large quantities of Rose chamber and cell strain (bottle) cultures. Six cine time-lapse, phase units are available and two still photomicrographic setups.
The professional group includes five investigators with doctor's degrees and eight technical assistants exclusively concerned with research projects.

9. Additional Requirements:

The work would be greatly hastened if we could add an assistant for chromosome counting. Dr. Nakanishi is certain that an ideal person for this could be obtained from Dr. Makino's group at Hokkaido University in Japan. This would regard as additional $5000.00

10. Additional Information (Including relation of work to other projects and other sources of supply):

We hope to examine the excellent fractionated preparations of tobacco tars which have been made by Mr. Jack Neal of the Department of Public Health and Preventive Medicine of this School.

It is hoped that our program may encompass studies on the smog problem so that valid comparisons may be made between this and the tobacco factor in relation to the activity of human pulmonary cells in vitro. It has been possible to prove in provisional work that lesions are produced in mitochondria both by smoke condensate and by ozone. The Smog Committee of the Los Angeles County Medical Society and Professor A. J. Haagen-Smit of California Institute of Technology have helped us with pilot studies. It is possible that additional funds may become available from a California group for this phase of the investigation.

Signature of Director of Project: /s/ C. M. Pomerat

Signature of Business Officer of the Institution: /s/ E. D. Walker

Source: University of California, San Francisco, Special Collections & Archives, MSS 2000/01

TIRC Grant #1348

[Reprinted from Texas Reports on Biology and Medicine, Volume 17, Number 3, pages 345–353, Fall, 1959]

ON THE CHROMOSOME NUMBERS OF HUMAN AMNION CELLS IN PRIMARY AND STRAIN CULTURES*

Y. H. Nakanishi[a], M. V. Fernandes[b], M. Mizutani[c] and C. M. Pomerat

Cell cultures from human amnion recently have attracted considerable attention. The report of the establishment of a cell which persisted as an easily manageable "strain" by Fogh and Lund (1957) was followed by descriptions of morphological events occurring in the course of the formation of other lines by Dunnebacke (1956). Fernandes (1958) launched a similar line with the important features that within 35 days it appeared to have acquired the rapidly proliferative and nutritionally facultative characteristics which are generally ascribed to this class of cells.

Amnion cell strains have been reported to be particularly useful for virological studies. Pomerat et al. (1958) suggested that giant cell formation in amnion and other cell lines as a result of irradiation might be related to the elevated number of chromosomes typical for some of the cells in this organ.

The present study was undertaken with the hope of providing a more detailed chromosomal analysis of primary and strain cultures of a human amnion than those reported in the previous papers (Fernandes, 1958; Pomerat et al., 1958) with a description of the morphology of their chromosomes.

Materials and Methods

Placental tissue from a healthy woman was obtained from the John Sealy Hospital in Galveston, Texas.

Test objects consisted of 3 types of amnion derivatives:

I. *Freshly isolated elements before culture* (cf., figs. 1–3). Using amnion obtained immediately upon delivery, about 150 fragments obtained from various areas measuring approximately 1 mm. on a side were employed for making crush preparations stained with acetic orcein after treatment with a hypotonic solution (1 part of Gey's balanced salt solution plus 9 parts of ordinary tap water) for one hour.

II. *Epithelial cells in primary cultures of amnion tissue.* After 25 days of incubation, cells on cover glasses in roller tubes which had not been freed

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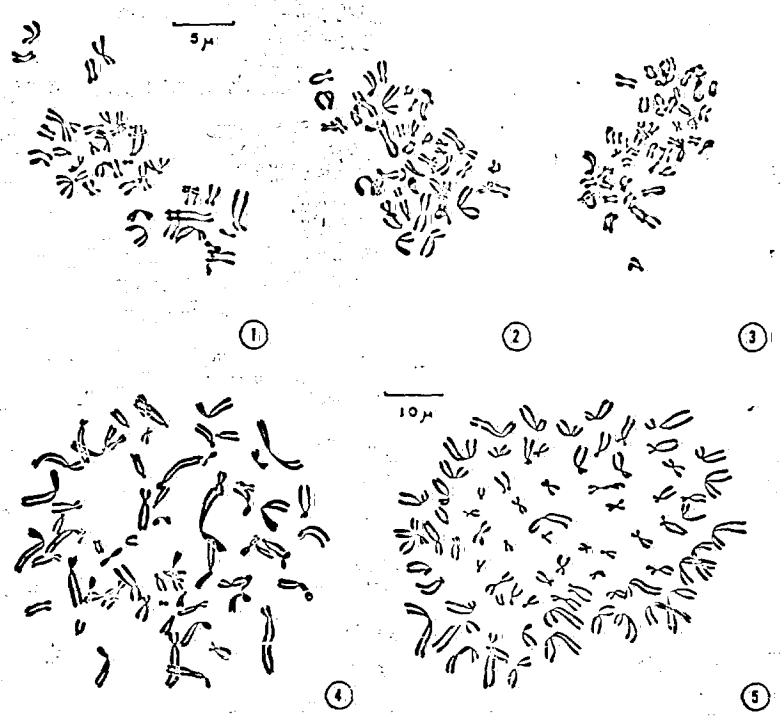
Source: https://www.tobaccodocuments.org/ltts/ttvm0000

with trypsin solution were treated with a hypotonic solution for 10 minutes, fixed with methyl alcohol and stained according to Jacobson's method (Hsu and Pomerat, 1953; Nakanishi, in press).

III. *The development of the Fernandes amnion strain of epithelial cells* (cf., figs. 4–5). This resulted from the trypsinization of amnion tissue and its cultivation without embryonic extract (Fernandes, 1958; Pomerat et al., 1958). On the 35th day from the date of the original trypsinization the chromosome numbers were counted in Rose chamber cultures of the 2nd passage using the same method as that applied for primary cultures. Later a newer technique as described by Hsu and Klatt (1958), Nakanishi and Mizutani (1959) and Nakanishi (in press) was utilized to observed the chromosomes of this strain.

Eagle's medium was employed with the addition of 10 per cent horse serum for primary cultures and for the establishment and maintenance of the strain. At the present time this strain is in the 97th passage, which is over two years after the date of the original trypsinization (April 1957 to May 1959).



FIGS. 1 to 5. Camera lucida drawings of the chromosomes of human amnion cells. FIGS. 1 to 3—freshly isolated cells before culture (42, 48 and 55 chromosomes, respectively). FIGS. 4 to 5—chromosomes of the strain cultures (67 chromosomes in the 2nd subculture and 75 elements in the 84th subculture). The magnification for FIGS. 1 to 4 is given by the scale shown in figure 1 and for figure 5 as shown.

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Source: https://www.biodiversitylibrary.org/; www.biodiversitylibrary.org/tfvm0000

Results and Discussion

I. *The chromosomes of freshly isolated amnion cells.* Walker (1958) found diploid, tetraploid and octoploid numbers of chromosomes in the transitional epithelium of the bladder of mice. For the bladder of newborn mice in which there was a wide range of nuclear size, he reported that out of 139 cells 68 were diploid, 63 were tetraploid and 8 were octoploid. It was concluded that polyploidy and large nuclei in newborn mice were related to normal differentiation by means of which "dome" cells in the surface layer of the transitional epithelium were produced. On the basis of both chromosome counts and modifications of nuclear size, Sachs and Shelesnyak (1955) studied the occurrence of polyploidy in the development of the deciduomata and its absence when this organ was suppressed byan antihistaminic agent. These authors concluded that polyploidy might be related to some specialized activity in the maternal placenta and that since these cells could not reproduce themselves normally or persist, this might provide a possible basis for the limited life of the deciduoma.

In a cytological study of the regenerating rat liver, Makino and Tanaka (1953) and Tanaka (1953) found that while there was a wide variation in the chromosome number of regenerating liver cells from adult rats during restoration after partial extirpation, the most frequent numbers fell in three ranges: diploid (36 to 46 chromosomes), triploid (59 to 68 chromosomes) and tetraploid (78 to 89 chromosomes). However, those in the diploid range were the most numerous.

In order to check the possibility that some areas of the human amnion normally contain cells with more than the diploid number, it was necessary to establish the chromosome count for freshly isolated elements before culture. Unfortunately, the amnion cells obtained directly from fetal membranes showed very little divisional activity. Only 3 cells revealed chromosomes sufficiently well spread to permit accurate counting. The values obtained were 42, 48 and 55 (Figures 1, 2, 3). Even though the chromosomes of only 3 cells were counted, this observation was suggestive of a diploid range for the human amnion.

II. *The chromosomal status of cells in primary cultures.* The chromosome numbers counted in both primary and strain cultures are presented in figure 6. Results for the 24 cells with well-

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Source: https://www.biodiversitylibrary.org/item/[illegible]

spread chromosomes obtained in material fixed on the 25th day of *in vitro* life showed a range from 41 to 52 with the highest values at 46 (25%) and 48 (21%).

Since Painter (1923) reported that the chromosome number in man was 48 and Oguma and Kihara (1923) stated that there were 47 chromosomes in the male, a number of workers have challenged these counts. The difficulties encountered in the enumeration of human chromosomes are obviously due mainly to the techniques associated with the handling of cells containing large numbers of these structures. The development of methods for the spreading of chromosomes in cell culture has made possible more critical studies of their number and morphology. Hsu (1952) reported 48 chromosomes in the material from tissue culture. However, he now states (Hsu, 1959) that even with hypotonic solution treatment which greatly enhances the spreading of the chromosomes, his original observation was incorrect. Recently, Tjio and Levan (1956) and Tjio and Puck (1958) found 46 chromosomes for cells of this species in tissue cultures. Further, Kodani (1958) reported that 46, 47 and 48 chromosomes occurred in testicular material. Nakanishi (in press) has studied the chromosome number of cells from human fetal lung tissue. In primary culture he found the most frequent chromosome numbers to be 46 (25%), 47 (22%) and 48 (22%). These values closely approximated those obtained in the course of the present study. Painter (post-graduate seminar, University of Texas, 1958) has discussed Kodani's suggestion that it seemed certain that humans might show either 46, 47 or 48 chromosomes, the variation in number probably being due to the presence of a definite chromosome pair, a univalent, or the absence of an entire pair and pointed out that chromosome "fusion" was quite common in nature.

The newer techniques which have been applied for the observation of chromosomes in tissue cultures (cf., Hsu and Klatt, 1958; Nakanishi and Mizutani, 1959; Nakanishi, in press and in this paper) may be helpful in settling the vexatious uncertainty of the somatic number for man.

III. *Data on chromosomes in strain cultures.* Counts made from 32 cells of the Fernandes amnion strain fixed on the 35th day of incubation had a range from 60 to 77 with a peak at 67 (subtriploid number). Cells in the 40th subculture (195 days

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Source: https://www.biodiversitylibrary.org/; http://www.biodiversitylibrary.org/item/tfvm0000

from the date of the initial trypsinization) showed a range from 58 to 90 with a peak at 66. In the latest observations of 50 metaphase plates in the 84th subculture (664 days) the most frequent chromosome number was 75 (31 out of 50 cells) in a narrower range of 69 to 78. In these subcultures the chromosome counts apparently were in the triploid range and no diploid number was found (Figure 6). Examples of excellent metaphase plates which facilitated the examination of chromosomes are presented for reference (Figures 4, 5, 7, 8).

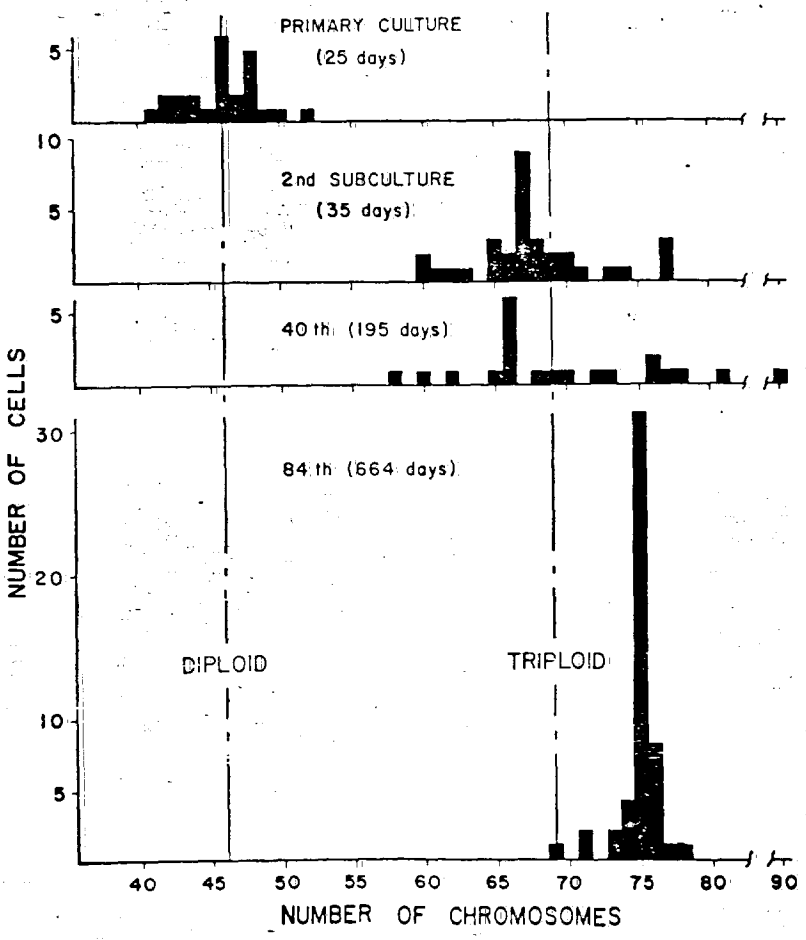

FIG. 6. Distribution of chromosome numbers in human amnion cells in primary and strain cultures.

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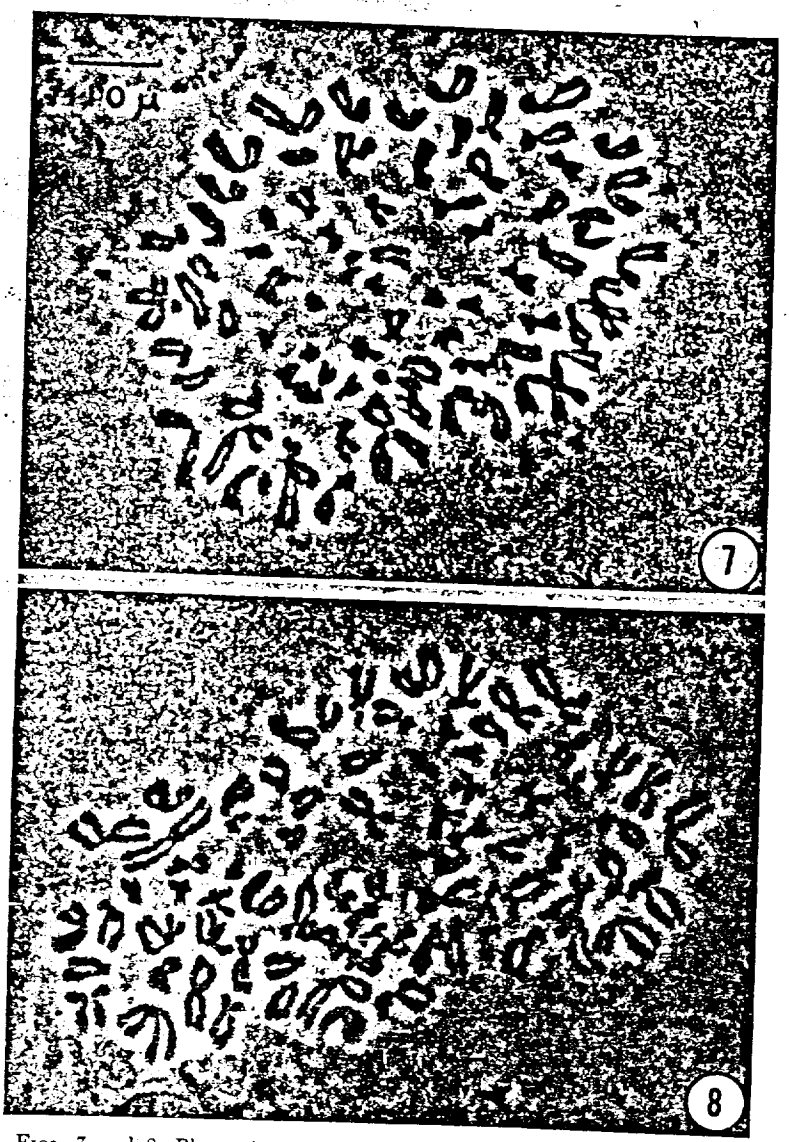
Source: https://www.tobaccodocuments.org/docs/tfvm0000



FIGS. 7 and 8. Photomicrographs of chromosomes of the human amnion cell strain (75 chromosomes, respectively). FIG. 7 corresponds to figure 5. The magnification for figures 7 and 8 is given by the scale shown in figure 7.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvh0000

As reported by many workers (cf. Levan, 1956; Hsu et al., 1957; Berman et al., 1957; Hsu and Klatt, 1959; Levan and Biesele, 1958; Nakanishi, in press) after continuous cultivation *in vitro*, cells of both normal and malignant origin exhibit extensive heteroploidy with very few or no euploids. Two human lung cell strains have been described (Nakanishi, et al.). The most frequent chromosome numbers were 76 and 77. In contrast, Syverton (1957) reported that in a strain of human tissue only diploids were found after many transfers. Recently, Tjio and Puck (1958) also stated that the chromosome number of cells from normal human skin, cervix and uterus remained constantly at 46 after more than 5 months of continuous, rapid growth in tissue culture involving scores of vessel transfers and a number of generations equivalent to many billions of progeny. Furthermore Ford and Yerganian (1958), in their studies on Chinese hamster cells, found that the diploids coexisted with the heteroploids for a long period.

Since the chromosome count made on the 35th day after the original trypsinization indicated a subtriploid number and also because the multiplicative rate of the cells was very high at this time, it was assumed that the strain was established before the 35th day (Fernandes, 1958). Levan and Biesele (1958) have reported that cells separated by trypsinization showed a higher incidence of chromosomal breaks than those which were mechanically minced. Further, they suggested that the reason trypsinization acted favorably in the establishment of new cell lines might be due to the appearance of cells with changed karyotype from the very beginning of tissue culture. The present observations would serve to supplement these data.

Summary

The chromosome numbers of human amnion cells were studied. The values obtained in freshly isolated elements before culture were 42, 48 and 55. The most frequent chromosome numbers in 25-day primary tissue culture preparations were 46 (25%) and 48 (21%). Counts made from cells of trypsinized cultures fixed on the 35th day of incubation (2nd subculture) had a range from 60 to 77 with a peak at 67. Cells in the 40th subculture (195 days from the date of the initial trypsinization) showed a range from 58 to 90 with a peak at 66. In the latest

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observations in the 84th subculture (664 days) of the Fernandes amnion strain, the most frequent chromosome number was 75 (62%) in a narrower range of 69 to 78. The problem of determining the precise somatic chromosome number for man is discussed.

Acknowledgments

Special thanks are due to Mrs. Walther Hild and Mr. Charles Raiborn for aid in the maintenance of the cultures and to Messrs. C. G. Lefeber and E. E. Pitsinger for the preparation of the photographs.

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b Present address: Laboratório Nacional de Investigação Veterinária, Lisbon, Portugal.

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TRC Grant #1343

OBSERVATIONS ON THE SOMATIC CHROMOSOMES OF LAMB CELLS IN CULTURE

By

YUH H. NAKANISHI AND MASAHIRO MIZUTANI

[Reprinted from TEXAS REPORTS ON BIOLOGY AND MEDICINE, Volume 17, Number 1, pages 134–141, Spring, 1959]

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OBSERVATIONS ON THE SOMATIC CHROMOSOMES OF LAMB CELLS IN CULTURE[ab]

YUH H. NAKANISHI[c] AND MASAHIRO MIZUTANI[d]

The number of chromosomes reported by various workers for domestic sheep (*Ovis aries*) ranges from 33 to 60. Berry (1941), Ahmed (1940) and Makino (1943) have reported a diploid number of 54 chromosomes in the spermatogonial cells of the sheep. The present observations were undertaken with the hope of analyzing the somatic chromosomes of this species with the newer technique for the spreading of chromosomes in tissue culture.

Materials and Methods

Lung tissue from a 7-day-old male lamb (*Ovis aries*) was cut into fragments of approximately 1.5 mm. side length and explanted in plasma clots on 12 x 50 mm. cover slips of No. 1 thinness. The clot consisted of equal parts of heparinized rooster plasma and extract from 8-day chick embryos. Three explants were placed on each cover slip and after the clot became firm, these were placed in test tubes. Three hours after explantation 2 ml. of Eagle's medium with 10 per cent horse serum were added to each tube. The cultures were incubated at 37° C. and rotated at 8 revolutions per hour. The fluid medium was changed on the 4th day after explantation. The technique of preparing slides for chromosome studies was based upon the method used by Hsu and Klatt (in press) with some modifications. On the 7th day the cultures were treated with a colchicine solution (0.04 ml. of a 1:10,000 dilution of colchicine in distilled water plus 2 ml. Eagle's medium) for 16 hours at 37° C. At the end of this period the cultures were treated with a hypotonic solution (sodium citrate 1.12 per cent in distilled water) for 10 minutes at room temperature. They were then fixed in Carnoy's fluid for 5 hours and stained slightly with diluted acetic orcein (0.2 gm. of orcein in 100 ml. of 50 per cent acetic acid) for ten minutes. The coverslips were extracted from the roller tubes and the explants were removed with the aid of a forceps. The coverslips carrying the cell outgrowth were inverted on to clean glass slides. They were covered with blotting paper and pressed slightly with the thumb. Finally the edge of the coverslips were sealed with Deckglasskitt nach Krönig.[e] A total of 75 explants was employed to observe the chromosomes. Observations were made with the use of a Zeiss phase contrast microscope (dark contrast 100x objective and a Zeiss 8x ocular), and a Bausch and Lomb phase contrast microscope (dark contrast 97x objective and a 12x ocular). Photomicrographs were made with a Spencer phase contrast microscope. The optical combination used consisted of a bright high contrast 97x objective and a Leitz 10x ocular.

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Observations

In the zone of outgrowth from each explant the cells were typical spindle-shaped and presumed to be fibroblasts, although in some cultures they were flattened and resembled epithelium. Accurate chromosome counts were made from drawings of 50 well spread metaphase plates. Results are presented in table 4 and figure 1.

TABLE 1

Distribution of chromosome numbers and of the numbers of cells with V-shaped elements. Lung of the lamb fixed 8 days after explantation.

Chromosome numbers:		46	47	48	49	50	51	52	53	54	55
Number of cells	Total	1	1	..	3	1	1	3	3	35	2
	5 V	..	1	..	1	1	..	..	..	2	..
	6 V	1	..	..	2	..	1	3	3	33	2

Chromosome numbers were in the range of 46 to 55 with a modal value of 54 (70 per cent) and in a series of 33 out of 35 cells of this type, there were 6 large V-shaped chromosomes.

Microscopical examination was made of the individual chromosomes to identify homologous pairs as accurately as possible. Each chromosome was drawn with the aid of a camera lucida with special attention being given to size and shape (Figs. 2, 3). The drawings were then photographed and from prints individual chromosomes were cut out with the aid of a pair of scissors. These were placed in serial alignment according to size and the locus of the centromere. Two such ideograms are shown in figures 6 and 7. The serial alignment of the chromosomes as given in these figures indicates that there are 26 pairs of autosomes and the sex pair which consists of unequal X and Y elements. The first three pairs of autosomes are especially long, being V-shaped with metacentric centromeres. Among the fourth to twenty-sixth pairs there are at least 20 to 22 which are of the J-shaped type. These apparently have subterminal centromeres showing a tiny globular segment at one end, while the remainder are rod-shaped. The sex chromosomes are represented by a long J-shaped element, the X, and an extremely small grain-like body, the Y. The X element seems to have a length lying approximately between that of the sixth and seventh autosome.

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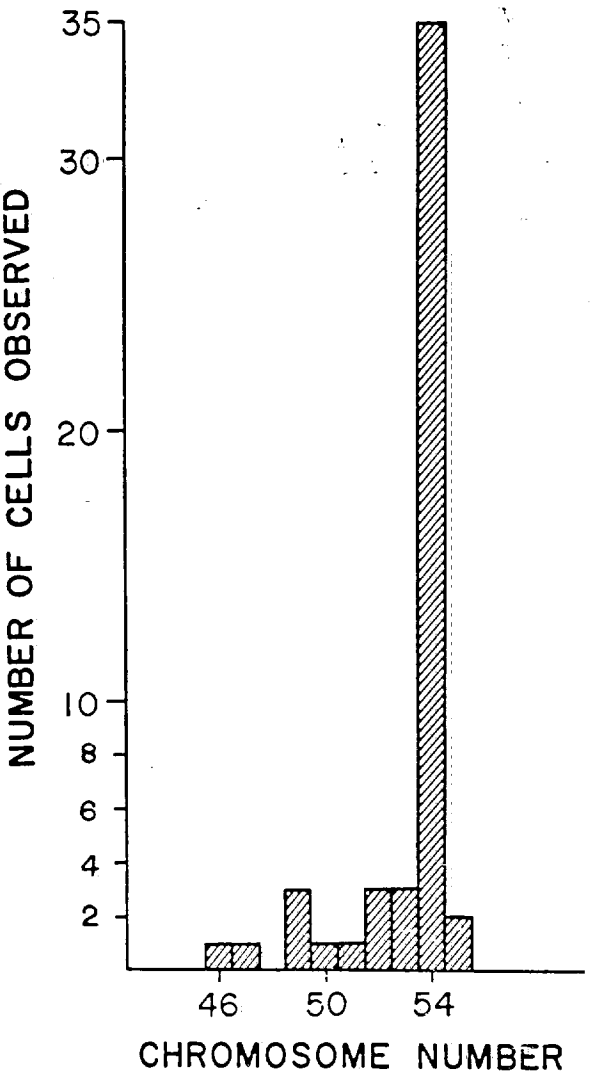

FIGURES 1 to 7 were based on cells in cultures of lung cells from a male lamb treated with colchicine on the 7th day and fixed on the 8th day of incubation.

FIG. 1. Distribution of chromosome number in lamb lung tissue. A total of 50 well spread figures were observed.

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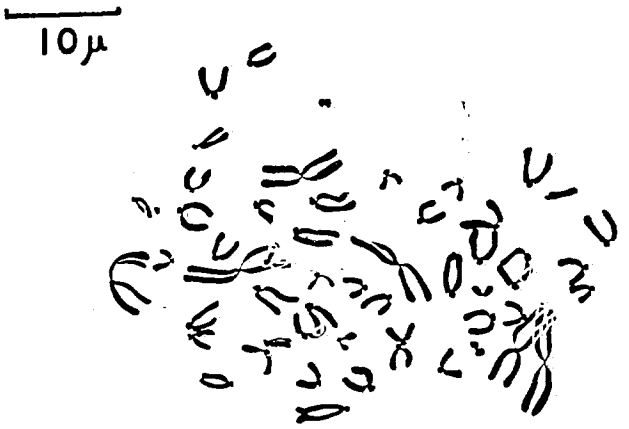
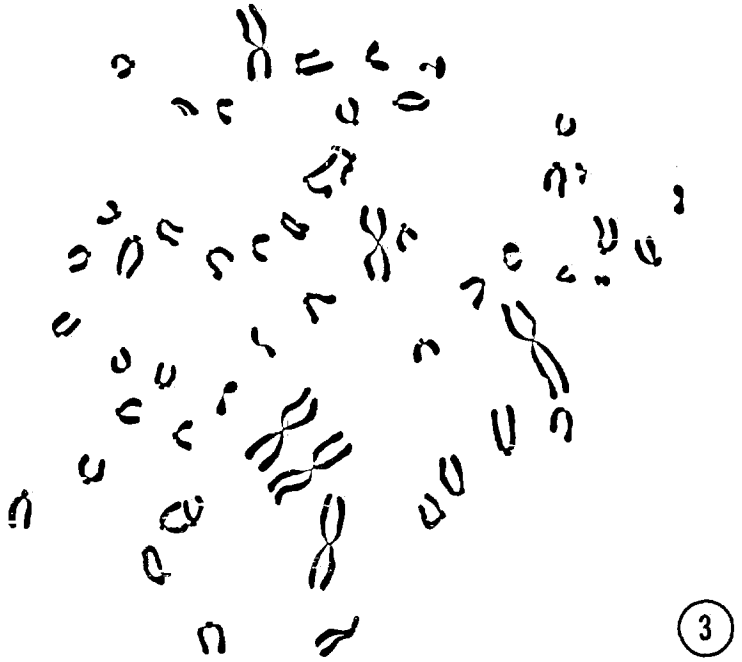


2



3

FIGS. 2 and 3. Camera lucida drawings of somatic chromosomes. The magnification for both drawings is given by the scale shown in figure 2.

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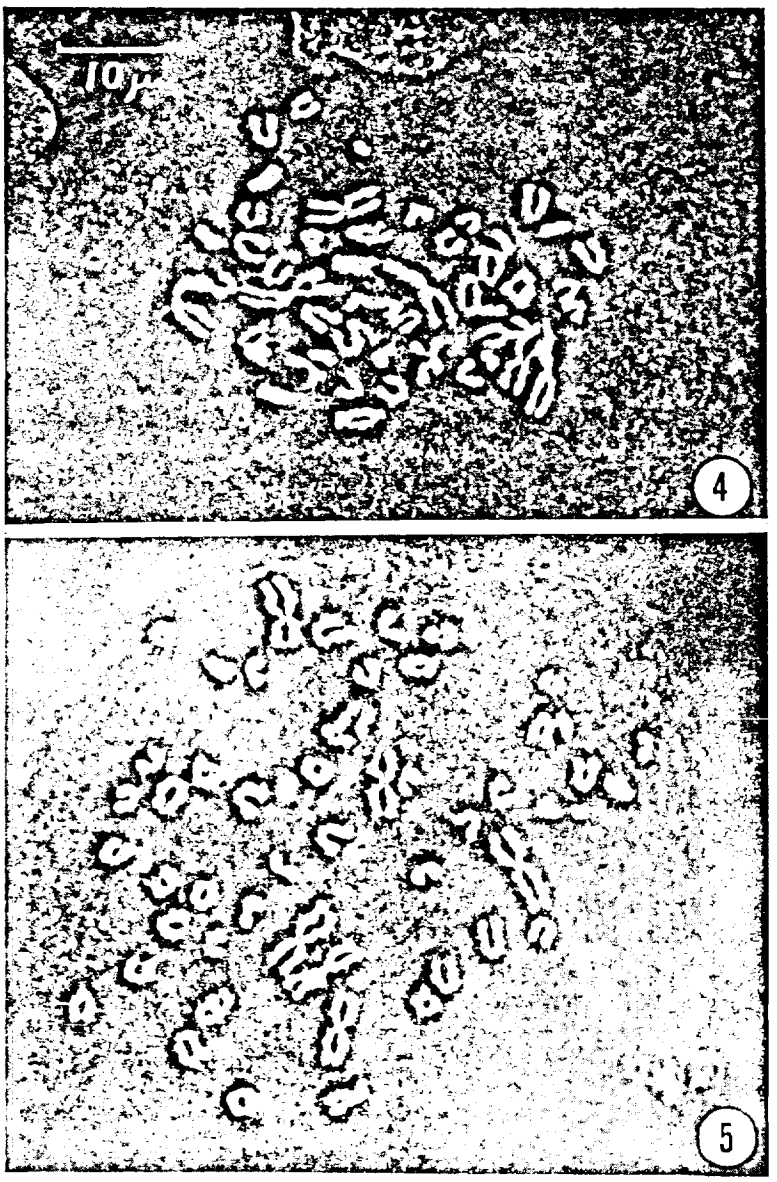

FIGS. 4 and 5. Photomicrographs of somatic chromosome. Figures 4 and 5 correspond to figures 2 and 3, respectively. The magnification for both photomicrographs is given by the scale shown in figure 4.

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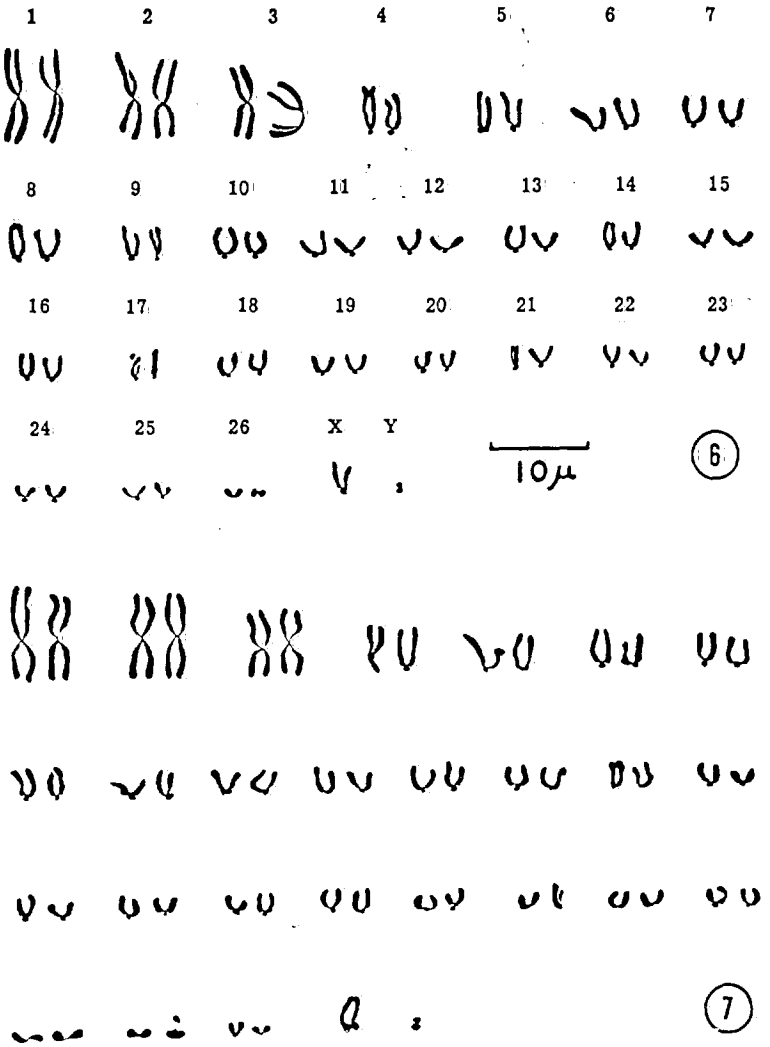

FIGS. 6 and 7. Serial alignments of chromosomes of the lamb in pairs which are presumed to be homologous in approximate order of size. Figures 6 and 7 correspond to figures 4 and 5, respectively. The magnification for both ideograms is given by the scale shown in figure 6.

Discussion

With regard to the chromosome number and in relation to some other morphological aspects, the result of the present study is in agreement, though not entirely, with the accounts given by

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Berry (1941) and Makino (1943). Ahmed (1940) reported the same number of chromosomes, but he stated that there were three large V-shaped chromosomes in the diploid set and that one of these was considered to be the X element.

Berry (1941) and Makino (1943) reported that the diploid complement of the sheep consisted of three homologous pairs of V-shaped chromosomes, 23 homologous rod-shaped pairs occurring in a gradual series and an unequal pair of the X and Y. The present observations, however, demonstrate that in the material obtained from the lung tissue of the lamb there are three pairs of V-shaped chromosomes and at least 20 to 22 pairs of elements having subterminal centromeres. The X element also carries this type centromere. The differences between the morphological data reported in this study and in those of previous reports (Berry 1941, Makino 1943) are obviously due mainly to the technical difficulties associated with the handling of cells containing large numbers of chromosomes. The development of the technique for the spreading of chromosomes in cell culture (Hsu and Pomerat, 1953) has made possible a more critical study of chromosome numbers and morphology.

For example, it had been thought on the basis of studies on germ cells that all rat chromosomes were rod-shaped elements having terminal centromeres until recent studies indicated that there are at least several pairs having subterminal and submedian centromeres according to the application of more modern techniques (Makino and Hsu, 1954; Ohno and Kinoshita, 1955; Tjio and Levan, 1956; Makino and Sasaki, 1958). As pointed out by Ohno and Kinoshita (1955) and Makino and Sasaki (1958), it seems to be possible, with further technical improvements, to establish that almost all elements of telomitic sets in various animal chromosomes are of acrocentric nature.

In the course of studies on the effect of tobacco condensates on human lung cells *in vitro*, the authors found shifts in the modal value and the development of dicentric chromosomes (unpublished). For such investigations the chromosome complex of lamb cells, even though involving large numbers, might prove useful in describing changes not only in chromosome counts but also in their shape because the peculiar combination of 3 long characteristic V-, 20–22 small J- and 1–3 rod shaped pairs might be traced more accurately.

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Summary

The somatic chromosomes of lung cells from a male lamb were studied in 8-day primary tissue culture preparations, since it is well established that the range of aneuploidy increases in the course of *in vitro* life. The most frequent chromosome number was determined as 54 (35 out of 50 cells). Morphological analysis revealed that the diploid complement of the lamb consists of 6 large V-, 40 to 44 J- and 2 to 6 rod-shaped autosomes. The X chromosome is J-shaped and the Y element is of the rod type.

Acknowledgments.

The authors are deeply indebted to Professor S. Makino, Zoological Institute, Hokkaido University, Sapporo, Japan, for his inspiration and to Dr. C. M. Pomerat for his valuable advice in the preparation of the manuscript. Mrs. Walther Hild and Mr. Charles Raiborn aided in the setting up of the cultures and Messrs. C. G. Lefeber and E. E. Pitsinger assisted with the preparation of photographs.

REFERENCES AND NOTES

For the older literature concerning the chromosomes of the sheep and rat other than those noticed below, reference is made to Makino 1951, 1956: *An Atlas of the Chromosome Numbers in Animals*, Iowa State College Press, Iowa, and the revised edition, Tokyo, pp. 172–173, 176, 294–297.

Ahmed, I. A. The structure and behavior of the sheep during mitosis and meiosis. *Proc. Royal Soc.*, Edinburgh, 60: pp. 260–270, 1940.

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[a] Aided in part by a grant from the American Cancer Society administered by C. M. Pomerat. Paper accepted December, 1958.

[b] Tissue Culture Laboratory, The University of Texas, Medical Branch, Galveston.

[c, d] Fellows of the Tobacco Industry Research Committee. Permanent addresses: Zoological Institute, Hokkaido University, Sapporo, Japan.

[e] This wax can be obtained from Riedel Haën A.-G., Seelze bei Hannover, Germany.

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#134BR3

Committee:
Little
Kotin
Jacobson

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

Activated 1/1/57
Renewed 1/1/58
" 1/1/59

Renewal Application for Research Grant

Date: November 23, 1959

1. Name of Investigator: C. M. Pomerat, Ph.D.

2. Title: Professor of Cytology, Department of Anatomy, - Director, Tissue Culture Laboratory

3. Institution & Address: The University of Texas - Medical Branch Galveston, Texas

4. Project or Subject: Tissue culture studies of human lung.

5. Detailed Plan of Procedure: Using both experimental animals and human cells, we would like to continue studying the effect of various noxious chemicals on chromosomal patterns. We would like to continue checking the suggestion that components of tobacco tars may act as co-carcinogens.

Animals are being used to determine whether cells with experimentally produced dicentric chromosomes are capable of inducing neoplasms. A cell line established from a tobacco induced mixed tumor has been made to yield several "clones." Further studies of the chromosome numbers and the tumor producing propensities of pure clone lines are urgently needed.

Techniques evolved for the analysis of smoke injury to chromosomal patterns should be extended to air pollutants (smog) for comparative studies. Similarly, the effect of radiant energy on cell lines should be included in a broad program of assessing environmental factors which may contribute to the aetiology of pulmonary neoplasia.

6. Budget Plan:

Salaries	$ 9,500.00
Expendable Supplies	189.00
Permanent Equipment	-----
Overhead	1,091.00
Other - Travel	1,220.00
	$12,000.00

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7. Anticipated Duration of Work: One year.

8. Facilities and Staff Available: Please see previous application for facilities available. Staff: Y. Ohnuki (beginning March 1, 1960). Another investigator to replace Dr. Mizutani.

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9. Additional Requirements:

Travel expenses for new worker. Round trip air ticket (trans-Pacific) and U.S. train travel in advance at approximately $1200.00.

10. Additional Information (Including relation of work to other projects and other sources of supply):

Our program has developed an important series of experimental techniques and constants which are available for the evaluation of constituents of "smog" and radiation factors which must be taken into account in the analysis of the role which smoking may play in the genesis of neoplasia. I have been invited to contribute to the program of the 3rd Annual Medical Research Conference of the California State Department of Public Health on December 9 and 10, 1959, regarding our research approach.

/s/ C. M. Pomerat, Director of Project

/s/ E. W. Walker, Business Officer

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Biology, Carcinogenesis & Tissue Culture

Committee:
Dr. Little
Dr. Jacobson
Dr. Kotin

#134BR4
Activated 1/1/57
Renewed 1/1/58, '59, '60

TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street New York 17, N.Y.

Renewal Application for Research Grant

Date: August 16, 1960

1. Name of Investigator: C. M. Pomerat, Ph.D.

2. Tital: Director of Biological Research

3. Institution & Address: Pasadena Foundation for Medical Research
99 North El Molino Avenue
Pasadena, California

4. Project or Subject: Tissue culture studies of human lung.

5. Detailed Plan of Procedure:

The methods which have been perfected are the now classic ones employed with cell cultures for quantitating:

1. chromosome breaks,
2. abnormal mitoses,
3. chromosome enumeration,
4. "Marker" chromosomes.

As indicated by the bibliography and by the oral report given in New York City in May, 1960, these methods have been employed for

a. Fundamental studies on the development of cell strains as test objects (Reference nos. 1 and 3).

b. Study of alterations in the chromosome number of stem line cells following chronic contact with tobacco tar fractions (Reference no. 2).

c. A parallel effect was demonstrated with irradiation (Reference no. 4).

d. The chromosomal configuration of a tobacco tar induced tumor was employed for an in vitro study which showed chromosomal changes at the near diploid level (Reference no. 5).

e. Assessing the relative toxicity of tobacco versus paper pyrolysates (Oral report - work in progress).

Future Plans

As stated by Heller on page 23 in "Progress Against Cancer - 1959" (P.H.Service Publication No. 738) "... In the opinion of many investigators, multiple factors are operative in the production of lung cancer." We believe that the methods listed from 1 to 4 as employed in our group by extraordinarily intelligent and skillful fellowship students trained by Professor S. Makino at the University of Hokkaido (Sapporo, Japan) should serve for multiple attacks in the study of atmospheric agents believed to exert noxious effects on cells. Specifically, in addition to tobacco and its by-products, pollutants derived from auto exhausts, soot particles, and airborne radioactive substances must be examined.

10035412720

References

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2. Nakanishi, Y.H., M. Mizutani and C.M. Pomerat: Smoke condensates on lung cells in tissue culture with special reference to chromosomal changes. Texas Rep. Biol. and Med. 17:542-590, 1959.

3. Nakanishi, Y.H.: The development of a kitten lung cell strain and its chromosomes. Zeit. f. Zellforsch. 51:138-151, 1960.

4. Mizutani, M., Y. Ohnuki, Y.H. Nakanishi and C.M. Pomerat: Changes in modal values of chromosomes following irradiation of human amnion cells. Cancer (in press).

5. Mizutani, M., Y. Ohnuki, Y.H. Nakanishi and C.M. Pomerat: The development of a near diploid in vitro strain from a smoke condensate induced mouse tumor. Texas Rep. Biol. and Med. (in press).

6. Budget Plan:

Salaries	$ 9,500.
Expendable Supplies	189.
Permanent Equipment	-
Overhead (11.3%)	1,091.
Other (Travel)	1,220.
Total	$12,000.

7. Anticipated Duration of Work: 2 years

8. Facilities and Staff Available:

As reported previously, we have maintained junior and senior research fellows on an overlapping 2-year basis. The Pasadena Foundation for Medical Research is described in the attached report. A new brochure is in the process of preparation which will be sent as soon as it is ready. Practically all the apparatus and equipment in the Galveston Tissue Culture Laboratory was transferred to Pasadena, together with 4 members of the technical staff. The total space which is new is somewhat greater here. The effort is strengthened by having Dr. Donald Rounds as co-director. Dr. S. Shankman is in charge of the work in organic synthesis and Dr. M. Roberts is the pharmacologist for our group.

9. Additional Requirements:

Dr. Y. Ohnuki will be returning to Japan April 30, 1961. We have been assured by Professor Makino that he can be replaced by a fellow of equal training and competence. Travel expenses for new worker - round trip air ticket (trans-Pacific) in advance at approximately $1,200.00.

10. Additional Information:

The Stanford Research Institute of South Pasadena has assured full cooperation in making auto gases and their fractionations available to us (Reference: Dr. L. Jones). Dr. H.L. Helwig of the Industrial Hygiene Laboratory of the State of California Department of Public Health has already helped us in obtaining smog samples from the Los Angeles area in the form of filter paper particulate collections.

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Dr. Cornelius Tobias of the Donner Laboratories has agreed to make radiation facilities on the University of California, Berkeley campus available to us for projects related to our contract with the U.S. Air Force.

It may be added humbly that the undersigned has been made Adjunct Professor of Anatomy at the Medical School of the University of Southern California and Clinical Professor of Pathology at the College of Medical Evangelists. A 12-week course on the "Principles and Methods of Tissue Culture" will be offered at the latter institution in the Spring of 1961.

Signature C. M. Pomerat
Director of Project

George S. Sharp
Business Officer of the Institution

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TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

106

Application For Research Grant

Date: July 20, 1955

1. Name of Investigator: Leon Pordy, M.D.

2. Title: Research Assistant in Cardiology

3. Institution & Address: The Mount Sinai Hospital
100th Street and Fifth Avenue
New York City 29, New York

4. Project or Subject: Clinical Evaluation of the Cardiovascular Effects of Tobacco Smoking

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Using the objective methods available clinically, it is planned to study the xx cardiovascular effects of tobacco in the normal population as compared with cardiac patients, especially those suffering from coronary artery disease. In this regard, the routine clinical examination will be supplemented by the electrocardiogram, "2-step" exercise tolerance tests and the ballistocardiogram - both before and after smoking.

Preliminary report has been presented at the January 1955 Clinical Research Meeting at the New York Academy of Medicine (reprints attached); one hundred cases were included in this study. It is proposed to extend these studies over larger numbers of patients and to evaluate the results obtained by proper follow-up investigation.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	CHIEF TECHNICIAN	$ 3,464.80
Expendable Supplies		235.20
Permanent Equipment		-
Overhead		555.00
~~Other~~	Per Annum	4,255.00
	Total	12,765.00

7. Anticipated Duration of Work: Three Years

8. Facilities and Staff Available: Lazar,
Associates: Louis Allen ~~Lazar~~ M.D. - Assistant in ~~Cardiology~~ Cardiology;
Kenneth Chesky, M.D. - Research Assistant in Cardiology;
Arthur M. Master, M.D. - Chief, Cardiographic Laboratory.

Ward, Clinic and Hospital Personnel are available for study.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

The study of coronary circulation in normals and cardiacs by the electrocardiogram, "2-step" tests and ballistocardiogram are current hospital projects. This new investigation will dove-tail into current ones insofar as permanent equipment is concerned. The urgent need is for technical help for the tobacco studies - for recording, mounting ~~and~~ and filing the tracings obtained.

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Signature Leon Pordy, M.D.
Director of Project

Sidney M. Samis, M.D., Acting Director
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

#230
(cf. #51R3
Activated 7/1/55
Renewed 7/1/56
" 7/1/57)

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET
NEW YORK 17, N. Y.

Application For Research Grant

Date: 20 February , 1959

1. Name of Investigator: H. R. Pratt-Thomas, M.D.

2. Title: Professor of Pathology

3. Institution & Address: Medical College of South Carolina
16 Lucas Street, Charleston, South Carolina

4. Project of Subject: Carcinogenesis in the Laboratory Animal: A comparison of species response to standarized sites of application.

5. Detailed Plan of Procedure:

A towering edifice of experimental data relative to animal carcinogenesis has been constructed over the years, but whether this edifice is structurally sound, particularly in regard to its human application is open to question. The mouse and the rat have been the prime targets in these investigative efforts and the skin and subcutaneous tissues the favorite sites of endeavor. After a decade of working with mice as test animals in attempt to evaluate the possible carcinogenic effects of smegma and tobacco tars, it has become apparent that the murine cellular response is very labile (specifically as regards cervical and vaginal epithelium) and that a variety of stimuli of relatively inocuous proportions are capable of producing neoplasms e.g. simple trauma.
Previous Publications:
Heins, Henry C., Dennis, E. J., and Pratt-Thomas, H. R. : The Possible Role of Smegma in Carcinoma of the Cervix. Amer. Jour. Obs. and Gyn. 76: 726-735, October, 1958.
Pratt-Thomas, H. R., Heins, H. C., Latham, E., Dennis, E. J., and McIver, F. A.: The Carcinogenic Effect of Human Smegma: An Experimental Study. Part I: Preliminary Report. Cancer, 9: 671-680, July-Aug., 1956.; Also Biologic Assay of Cigarette Smoke Tars (to be published).

As the premise of carcinogenic effect in man is often supported by such evidence as the production of neoplasms in the laboratory animal, it is extremely important that species variations be taken into consideration so that any carcinogenic effects may be placed in their true perspective.

We propose to utilize groups of different laboratory animals, rabbits, mice, guinea pigs, hamsters, and rats (monkeys also desirable, but expensive and definitely require long term exposure) and to expose each group

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

to identical agents by means of the same procedures. We would utilize chiefly intra-vaginal application technique with which we have had a great deal of experience, but plan to use skin and subcutaneous areas as well so that we may employ a greater range of substances without increasing the number of animals to impractical proportions. The agents to be used would include some of the patent polycyclic hydrocarbons, but particular attention would be given to such agents as ethyl alcohol, silver nitrate, paraformaldehyde, simple trauma, xylene, etc. The impression has been gained that mice (at least some strains) will react in a neoplastic fashion to a wide variety of stimulating or irritating agents, provided the stimulus is applied for a sufficient length of time.

6. Budget Plan:

Salaries	$ 7,000.00
Expendable Supplies	800.00
Permanent Equipment	100.00
Overhead (10%)	790.00
Other	000.00
Total	$ 8,690.00

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available: H. R. Pratt-Thomas, M.D. (principal investigator) No salary.

Animal quarters and cages are available as well as all the major technical equipment as may be found in a large and well equipped Department of Pathology. Animal caretakers and the technicians who will maintain a regular schedule of the various applications proposed in this work will be the major item of expense. The same technicians will also prepare all the histologic material as it becomes available.

9. Additional Requirements: None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The publication of the U. S. Department of Health, Education, and Welfare "Survey of Compounds which have been tested for Carcinogenic Activity" Supplement 1, 1957 lends support to some of the proposals and ideas expressed in the formulation of this project. The numerous articles dealing with animal carcinogenesis and the implication, directly or indirectly inferred, of its relation to neoplastic disease in man makes clarification of this problem mandatory.

/s./ H. R. Pratt-Thomas
Director of Project

/s./ L. W. Michaelis
Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#50JR2
Activated: 2/1/55
Renewed: 2/1/56

RENEWAL

Application For Research Grant

Date: January 18, 1957

1. Name of Investigator: H. R. Pratt-Thomas, M.D.

2. Title: Professor of Pathology

3. Institution & Address: Medical College of South Carolina
16 Lucas St.
Charleston, S. C.

4. Project or Subject: Cooperative pathologic-anatomic research project.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed): According to the plan developed at the December second meeting of pathologists.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	$ 2,231.00
Expendable Supplies	250.00
Permanent Equipment	100.00
Overhead	286.00
Other	
Total	$ 2,867.00

7. Anticipated Duration of Work: June 30, 1957.

8. Facilities and Staff Available:

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of supply):

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Signature /S./ H. RAWLING PRATT-THOMAS, M.D.
Director of Project

/S./ L. W. MICHAELIS
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries
Expendable Supplies
Permanent Equipment
Overhead
Other
Total

7. Anticipated Duration of Work:

8. Facilities and Staff Available:

9. Additional Requirements:

10. Additional Information (Including relation of work to other projects and other sources of support):

Signature
Director of Project

Business Officer of the Institution

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

No classified

51

Application For Research Grant

Date: December 1, 1954

1. Name of Investigator: H. R. Pratt-Thomas, M. D.

2. Title: Professor Pathology
Medical College of South Carolina

3. Institution & Address: Medical College of South Carolina
Charleston, South Carolina

4. Project or Subject: Biological Assay of Cancer Producing Factors in Cigarette Smoke Tars

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

It is proposed that tars procured from cigarette smoke be applied to the cervix of the uterus of mice according to a technique that has been devised by this investigator. This technique was reported in a paper entitled: "Carcinogenic Effect of Human Smegma; an Experimental Study. A Preliminary Report" read before the American Cancer Society on October 18, 1954. (See attached abstract) This report concerns the experimental production of epidermoid carcinoma of the cervico-vaginal area of mice in a manner that should be practical for comparable application of such substances as the tars or extractives from cigarette smoke. From the results encountered in the previous experiment it appears that the cervix and contiguous vagina of mice is a susceptible area for the production of experimental surface epithelial growth. The nature of the epithelial surface of that location would appear to be more comparable to that of the respiratory area than an outer skin suface. It is proposed to use substances obtained from cigarette smoke in ways that would be approved by the TIRC Scientific Advisory Board and that these substances would, if possible, be obtained frm such sources as may be approved or certified by the Scientific Advisory Board as produced out of methods that may be judged comparable to natural cigarette smoking.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Salaries:

Research Assistant	$3000.00
Technician	2700.00
Animal Caretaker	1200.00

Expendable Supplies: $275.00 mice, food, histologic material, photography, etc.

Permanent Equipment: $220.00 plastic animal cages, slide-file, mouse speculums, etc.

10035417781

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

THE CARCINOGENIC EFFECT OF HUMAN SMEGMA: AN EXPERIMENTAL STUDY

A Preliminary Report*

By

H. R. Pratt-Thomas, M. D., and Henry C. Heins, M. D., Eugene Latham, M. D., E. J. Dennis, M. D., Forde A. McIver, M. D.

From the Department of Pathology of the Medical College of South Carolina, Charleston, South Carolina

* This investigation was supported by a research grant #C-1418 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

1003541782

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

ABSTRACT

An experimental project was begun in 1950, in an attempt to determine whether whole human smegma or any of its component parts are definitely carcinogenic. Smegma has been implicated as a possible causative agent of at least two forms of human carcinoma by statistical inference gathered from various social and racial groups showing variation in physical and hygienic practices. The rarity of carcinoma of the cervix among Jewish women and the virtual absence of carcinoma of the penis among individuals circumcized early in life furnish the two outstanding examples of carcinoma in which a causative relationship to smegma may be entertained.

A paste made from whole human smegma has been applied to the cervico-vaginal area of mice of the dba strain. This has been performed in two ways. A metal vaginal speculum has been devised for the introduction of smegma or other substances into the mouse vagina. Through this is inserted an obturator having a cup end which holds a "dose" of the material which is to be applied. The other method consists of placing smegma or its components in the mouse vagina and then closing the vaginal orifice with a subcuticular circular suture. Malignant neoplasms have resulted in the cervico-vaginal area of these mice following the use of whole human smegma in each of the techniques described above. The most impressive results were in a series of fifteen mice in which human smegma was applied intravaginally twice weekly. Of the twelve animals in this series which could probably be evaluated at the time of death or their being sacrificed, there were four overt invasive carcinomas, one early carcinoma with borderline invasion, and another showing changes indistinguishable from intra-epithelial carcinoma. The remaining six mice all showed marked epithelial hyperplasia of the cervico-vaginal mucosa. The injections in the six mice showing neoplastic changes were carried out over an average length of time of nineteen and one-half months, and had an injection average of ninety-nine.

1003541783

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

The minimum time and number of injections required to produce carcinoma was fourteen months and seventy-eight injections. It was hypotheticated by us that the bacterial action in decomposing smegma might transform the cholesterol content of smegma into a carcinogenic agent.

Smegma has been analyzed from the bacteriological and chemical standpoint, and these various components are being tested at the present time alone and in combination with other factors such as estrogens. The contents of human ovarian dermoid cysts have been employed as control material.

100354178

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan

Salaries	~~$6,900.00~~
Expendable Supplies	~~275.00~~
Permanent Equipment	~~220.00~~
Overhead	~~739.50~~
Other	
Total	~~$8,134.50~~

7. Anticipated Duration of Work: ~ 3 years

8. Facilities and Staff Available: H. R. Pratt-Thomas (Principal Investigator) ~ No Salary

We have a large and active Department of Pathology. All the usual major technical facilities are available such as microscopes, microtomes, tissue processing apparatus, etc. There are at least three major experimental projects supported by Grant-in-Aids.in progfess at the present time, two of which are under my direction.

The major expenses concern competent help, as furnished by a research assistant, technician (who would also handle records and minor secretarial work) and an animal caretaker.

9. Additional Requirements:

None

10. Additional Information (Including relation of work to other projects and other sources of supply):

It will be noted that it is proposed that such tars as may be secured from cigarette smoke by methods or from sources judged suitable and relevant by the TIRC Scientific Advisory Board in accordance with the adopted program of the Board.

100354285

Signature /S/H. Rawling Pratt-Thomas
Director of Project

/S/ M. Fairley
Business Officer of the Institution

Source: https://www.industrydocuments.ucsf.edu/docs/tfvn0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#51R1

Application For Research Grant/ RENEWAL

Date: April 27, 1956

1. Name of Investigator: H. R. Pratt-Thomas, M.D.

2. Title: Application of a New Bio-Assay Technique in Examination of Cigarette Smoke Condensate for Possible Carcinogens"

3. Institution & Address: Medical College of South Carolina
16 Lucas St., Charleston, South Carolina

4. Project or Subject: See # 2 above

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

See Progress Report.

1003541786

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries	$6,900.00
Expendable Supplies	275.00
Permanent Equipment	220.00
Overhead	739.50
Other - Travel	500.00
Total	$8,634.50

7. Anticipated Duration of Work: Three years.

8. Facilities and Staff Available: See previous application.

9. Additional Requirements: None.

10. Additional Information (Including relation of work to other projects and other sources of supply): None

100354787

Signature

Director of Project

/s/ H. R. Pratt-Thomas, M.D.
Professor of Pathology
Medical College of S.C.

Business Officer of the Institution

/s/ L. W. Michaelis
Secretary-Treasurer, Medical College of S.C.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TIRC Grant #1 Progress Report #1

H.R. Pratt-Thomas, M.D. April 19, 1956

Medical College of South Carolina

CONFIDENTIAL

APPLICATION OF A NEW BIO-ASSAY TECHNIQUE IN EXAMINATION OF CIGARETTE SMOKE CONDENSATE FOR POSSIBLE CARCINOGENS.

103 mice of dba #1 strain have been used in this project thus far.

22 mice have received semi-weekly applications of tobacco tar condensate which is applied directly to the cervix by means of a cotton-tipped applicator saturated with the above solution.

10 mice have been subjected to a similar procedure at the same time intervals, but with the applicator saturated with solvent "A" (Vehicle in which the tobacco tar condensate has been dissolved).

Another 10 mice have been used in the same fashion using a dry cotton applicator.

The latter group serves as a control.

Of the remaining mice all have been used for insertion of cotton wicks. These wicks are first saturated with either the tobacco tar condensate or solvent "A" and are inserted into the cervical os. The wick consists of #8 cotton thread with a barrel-knot placed in one end. The end consisting of the knot is saturated with the appropriate solution before insertion. The free end is brought out of the uterine corpus and affixed to the abdominal wall so as to keep the saturated barrel-knot in close proximity to the cervical os.

Dry untreated cotton wicks have been used in another group to serve as a control.

- RESULTS -

A number of the mice used in the applicator groups have developed inflammatory lesions of the perineum with marked overgrowth of granulation tissue. As soon as lesions were noted the affected mice were isolated and applications discontinued. All lesions have healed spontaneously. It is interesting to note that most of the inflammatory lesions have developed in the solvent "A" applicator group. One inflammatory lesion occurred in the tobacco tar applicator group and one lesion occurred in a mouse of the group awaiting wick insertion and occurred prior to any of our manipulative procedures. This last mouse experienced the most severe lesion of all and resulted in marked perineal deformity due to the scarring, so that there is some question as to exact cause of these lesions.

We have not had any demonstrable neoplasms in the applicator group as yet. If our estimations are correct, based on previous research findings with carcinogens, this group would not be expected to develop neoplasms in less than 12 months, even if a proven carcinogen was being employed.

We believe that the above methods will enable us to eventually express an opinion as to the effects of tobacco tar condensate on the cervical and vaginal epithelium of mice and to evaluate any stimulatory or even carcinogenic properties that it may possess. The animals have simply not been committed for a sufficient length of time to make this possible at present.

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Document is available at the Industry Documents Library http://www.industrydocuments.ucsf.edu/docs/jrvh0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N. Y.

#5182

Application For Research Grant

(Activated 7/1/55
Renewed as of 7/1/56)

Date: 22 April 1957

1. Name of Investigator: H. R. Pratt-Thomas, M.D.

2. Title: Professor of Pathology
Medical College of South Carolina

3. Institution & Address: Medical College of South Carolina
16 Lucas Street, Charleston, South Carolina

4. Project or Subject: Biological Assay of Cancer Producing Factors in Cigarette Smoke Tars.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

It is proposed that tars procured from cigarette smoke be applied to the cervix of the uterus of mice according to a technique that has been devised by this investigator. This technique was reported in a paper entitled: "Carcinogenic Effect of Human Smegma; an Experimental Study". A Preliminary Report read before the American Cancer Society on October 18, 1954. (See attached abstract). This report concerns the experimental production of epidermoid carcinoma of the cervico-vaginal area of mice in a manner that should be practical for comparable application of such substances as the tars or extractives from cigarette smoke. From the results encountered in the previous experiment it appears that the cervix and contiguous vagina of mice is a susceptible area for the production of experimental surface epithelial growth. The nature of the epithelial surface of that location would appear to be more comparable to that of the respiratory area than an outer skin surface. It is proposed to use substances obtained from cigarette smoke in ways that would be approved by the TIRC Scientific Advisory Board and that these substances would, if possible, be obtained from such sources as may be approved or certified by the Scientific Advisory Board as produced out of methods that may be judged comparable to natural cigarette smoking.

Signature /s/ H. R. Pratt-Thomas
Director of Project

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Industry Documents Library https://www.industrydocuments.ucsf.edu/docs/fvmn0000

6. Budget Plan:

Salaries	$ 6,900.00
Expendable Supplies	275.00
Permanent Equipment	80.00
Overhead	755.50
Other	300.00
Total	8,310.50

7. Anticipated Duration of Work: One additional year.

8. Facilities and Staff Available: H. R. Pratt-Thomas, M.D. (Principal Investigator) - No salary.

We have a large and active Department of Pathology. All the usual major technical facilities are available such as microscopes, microtomes, tissue processing apparatus, etc. There are at least five major experimental projects supported by Grant-in-Aids in progress at the present time, two of which are under my direction. The major expenses concern competent help, as furnished by a research assistant, technician (who would also handle records and minor secretarial work)and an animal caretaker.

9. Additional Requirements:

None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

It will be noted that it is proposed that such tars as may be secured from cigarette smoke by methods or from sources judged suitable and relevant by the TIRC Scientific Advisory Board in accordance with the adopted program of the Board.

Signature /s/ H. R. Pratt-Thomas
Director of Project

/s/ L. M. Michaelis
Business Officer of the Institution

Source: http://www.industrydocuments.ucsf.edu/docs/

#230
(cf. #51R3
Activated 7/1/55
Renewed 7/1/56
" 7/1/57)

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET
NEW YORK 17, N. Y.

Application For Research Grant

Date: 20 February , 1959

1. Name of Investigator: H. R. Pratt-Thomas, M.D.

2. Title: Professor of Pathology

3. Institution & Address: Medical College of South Carolina
16 Lucas Street, Charleston, South Carolina

4. Project of Subject: Carcinogenesis in the Laboratory Animal: A comparison of species response to standarized sites of application.

5. Detailed Plan of Procedure:

A towering edifice of experimental data relative to animal carcinogenesis has been constructed over the years, but whether this edifice is structurally sound, particularly in regard to its human application is open to question. The mouse and the rat have been the prime targets in these investigative efforts and the skin and subcutaneous tissues the favorite sites of endeavor. After a decade of working with mice as test animals in attempt to evaluate the possible carcinogenic effects of smegma and tobacco tars, it has become apparent that the murine cellular response is very labile (specifically as regards cervical and vaginal epithelium) and that a variety of stimuli of relatively inocuous proportions are capable of producing neoplasms e.g. simple trauma.
Previous Publications:
Heins, Henry C., Dennis, E. J., and Pratt-Thomas, H. R. : The Possible Role of Smegma in Carcinoma of the Cervix. Amer. Jour. Obs. and Gyn. 76: 726-735, October, 1958.
Pratt-Thomas, H. R., Heins, H. C., Latham, E., Dennis, E. J., and McIver, F. A.: The Carcinogenic Effect of Human Smegma: An Experimental Study. Part I: Preliminary Report. Cancer, 9: 671-680, July-Aug., 1956.; Also Biologic Assay of Cigarette Smoke Tars (to be published).

As the premise of carcinogenic effect in man is often supported by such evidence as the production of neoplasms in the laboratory animal, it is extremely important that species variations be taken into consideration so that any carcinogenic effects may be placed in their true perspective.

We propose to utilize groups of different laboratory animals, rabbits, mice, guinea pigs, hamsters, and rats (monkeys also desirable, but expensive and definitely require long term exposure) and to expose each group

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

to identical agents by means of the same procedures. We would utilize chiefly intra-vaginal application technique with which we have had a great deal of experience, but plan to use skin and subcutaneous areas as well so that we may employ a greater range of substances without increasing the number of animals to impractical proportions. The agents to be used would include some of the patent polycyclic hydrocarbons, but particular attention would be given to such agents as ethyl alcohol, silver nitrate, paraformaldehyde, simple trauma, xylene, etc. The impression has been gained that mice (at least some strains) will react in a neoplastic fashion to a wide variety of stimulating or irritating agents, provided the stimulus is applied for a sufficient length of time.

6. Budget Plan:

Salaries	$ 7,000.00
Expendable Supplies	800.00
Permanent Equipment	100.00
Overhead (10%)	790.00
Other	000.00
Total	$ 8,690.00

7. Anticipated Duration of Work: 3 years

8. Facilities and Staff Available: H. R. Pratt-Thomas, M.D. (principal investigator) No salary.

Animal quarters and cages are available as well as all the major technical equipment as may be found in a large and well equipped Department of Pathology. Animal caretakers and the technicians who will maintain a regular schedule of the various applications proposed in this work will be the major item of expense. The same technicians will also prepare all the histologic material as it becomes available.

9. Additional Requirements: None.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The publication of the U. S. Department of Health, Education, and Welfare "Survey of Compounds which have been tested for Carcinogenic Activity" Supplement 1, 1957 lends support to some of the proposals and ideas expressed in the formulation of this project. The numerous articles dealing with animal carcinogenesis and the implication, directly or indirectly inferred, of its relation to neoplastic disease in man makes clarification of this problem mandatory.

/s./ H. R. Pratt-Thomas
Director of Project

/s./ L. W. Michaelis
Business Officer of the Institution

10035417792

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

Bioassay, carcinogenesis and tissue culture #230R1

Committee:
Drs. Jacobson, Chm.
Reimann
Kotin
Little

Originally activated
Feb. 1, 1960

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 East Forty Second Street
New York 17, N.Y.

Application For Renewal of Research Grant

Date: October 31, 1960

1. Name of Investigator: H. R. Pratt-Thomas, M.D.

2. Title: Professor & Chairman of the Department of Pathology

3. Institution & Address: Medical College of South Carolina
16 Lucas Street, Charleston, South Carolina

4. Project or Subject: Carcinogenesis in the Laboratory Animal: A comparison of species response to standardized sites of application.

5. Detailed Plan of Procedure:

A towering edifice of experimental data relative to animal carcinogenesis has been constructed over the years, but whether this edifice is structurally sound, particularly in regard to its human application is open to question. The mouse and the rat have been the prime targets in these investigative efforts and the skin and subcutaneous tissues the favorite sites of endeavor. After a decade of working with mice as test animals in an attempt to evaluate the possible carcinogenic effects of smegma and tobacco tars, it has become apparent that the murine cellular response is very labile (specifically as regards cervical and vaginal epithelium) and that a variety of stimuli of relatively innocuous proportions are capable of producing neoplasms e.g. simple trauma and as supported by the following:

Previous Publications:

Heins, Henry C., Dennis, E.J., and Pratt-Thomas, H.R.: The Possible Role of Smegma in Carcinoma of the Cervix. Amer. Jour. Obs. and Gyn. 76:726-735, October, 1958.
Pratt-Thomas, H.R., Heins, H.C., Latham, E., Dennis, E.J., and McIver, F.A.: The Carcinogenic Effect of Human Smegma: An Experimental Study. Part I: Preliminary Report. Cancer, 9:671-680, July-Aug., 1956. Also Biologic Assay of Cigarette Smoke Tars (to be published).

As the premise of carcinogenic effect in man is often supported by such evidence as the production of neoplasms in the laboratory animal, it is extremely important that species variations be taken into consideration so that any carcinogenic effects may be placed in their true perspective.

1003541793

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

5. Detailed Plan of Procedure (continued-page 2)

We propose to utilize groups of different laboratory animals, rabbits, mice, guinea pigs, hamsters, and rats (monkeys also desirable, but expensive and definitely require long term exposure) and to expose each group to identical agents by means of the same procedures. We would utilize chiefly intra-vaginal application technique with which we have had a great deal of experience, but plan to use skin and subcutaneous areas as well so that we may employ a greater range of substances without increasing the number of animals to impractical proportions. The agents to be used would include some of the patent polycyclic hydrocarbons, but particular attention would be given to such agents as ethyl alcohol, silver nitrate, paraformaldehyde, simple trauma, xylene, etc. The impression has been gained that mice (at least some strains) will react in a neoplastic fashion to a wide variety of stimulating or irritating agents, provided the stimulus is applied for a sufficient length of time.

Report of Progress

It has been possible to obtain inbred strains of mice, rats, hamsters and guinea pigs.

One hundred and forty animals have now been committed to the experiment consisting of 30 mice, 50 rats, 30 hamsters and 30 guinea pigs. The animals have been divided into groups of ten and at the present time intravaginal application of the following substances are applied to the cervices twice weekly: 1% 3-4 Benzpyrene 15% 8-hydroxyquinoline, 1% tobacco tar concentrate. These materials have all been mixed in #1500 carbowax. We propose by January 1, 1961 to also utilize some unusual fatty acids obtained from various strains of mycobacteria as well as tryptophan metabolites. These will be utilized for intravaginal application and will, also along with the substances previously mentioned, be injected into the subcutaneous areas of these animals. Only a single subcutaneous injection of the material is contemplated at present. These materials have been chosen not only to test and compare the species response as originally proposed but also to possibly shed light on the problem of carcinogenesis in regards to chemical agents which we and other groups of investigators are studying.

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6. Budget Plan:

a.	Salaries	$7000.00
b.	Expendable Supplies	700.00
	Permanent Equipment	300.00
	Overhead (15% of a + b)	1155.00
	Other	-----
		$ 9155.00

7. Anticipated Duration of Work: 3 years.

8. Facilities and Staff Available: H. R. Pratt-Thomas, M.D. - Principal Investigator. No salary. R. R. Steuer, M.D. - Research Fellow in Pathology. James McInnes, Mack Davis and Ralph Mollycheck-Student Assistants. Frank Nelson-Diener. Miss Mildred Goddard-Technician.

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

8. Facilities and Staff Available:(Continued)

A first class temperature controlled animal room with proper equipment is in use. All technical pathology equipment is available and being utilized. Sufficient personnel to make the injections and properly care for these somewhat temperamental animals is the major item of expense.*

9. Additional Requirements:

Cages. See above.

10. Additional Information (Including relation of work to other projects and other sources of supply):

The publication of the U.S. Department of Health, Education, and Welfare "Survey of Compounds which have been tested for Carcinogenic Activity" Supplement 1, 1957 lends support to some of the proposals and ideas expressed in the formulation of this project. The numerous articles dealing with animal carcinogenesis and the implication, directly or indirectly inferred, of its relation to neoplastic disease in man makes clarification of this problem mandatory.

/s/ H. R. Pratt-Thomas, M.D., Director of Project

/s/ L. W. Michaelis, Business Officer of the Institution

* The cost has increased slightly from last year because of the number of animals being utilized and because a few additional cages are necessary.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

TOBACCO INDUSTRY RESEARCH COMMITTEE
150 EAST FORTY SECOND STREET NEW YORK 17, N.Y. #146

Application For Research Grant

Date: January 7, 1957

1. Name of Investigator: Richard C. Proctor, B. S., M.D.

2. Title: Assistant Professor of Psychiatry and Neurology

3. Institution & Address: Bowman Gray School of Medicine of Wake Forest College. Winston-Salem, North Carolina.

4. Project or Subject: An investigation of the emotional development of excessive smokers.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

An investigation to correlate the psychological, the psychiatric aspects of the personality of the excessive smoker. A background history and psychiatric evaluation of each individual excessive smoker will be obtained. This will be correlated with a complete battery of psychological tests. The main purpose of the investigation would be to discover what factors in common exist between excessive smokers from the standpoint of personalities, their reaction to emotional stress, and the liability of their mood swings. From a psychiatric standpoint it would appear that excessive smoking is an indication and result of anxiety and emotional insecurity rather than the cause of it. It would further appear that hypertension and tachycardia might be due in part to the same emotional factors that produce the excessive smoker. Also a correlation would have to be done between smokers and non-smokers with emotional instability.

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Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

6. Budget Plan:

Salaries		~~$20,750.00~~
Expendable Supplies		~~1,000.00~~
Permanent Equipment		~~1,000.00~~
Overhead		~~750.00~~
Other		~~1,500.00~~
	Total	~~$ 25,000.00~~

7. Anticipated Duration of Work: Twelve months.

8. Facilities and Staff Available: A psychiatrist is available on part-time basis to direct the project. A psychologist is available if funds are as is secretarial help and a psychiatric social worker.

9. Additional Requirements: It is anticipated that there will be no additional requirements.

10. Additional Information (Including relation of work to other projects and other sources of supply):

100354797

Signature /s/ Richard C. Proctor, M.D.
Director of Project

/s/ U. O. Parker.
Business Officer of the Institution

Controller

Source: https://www.industrydocuments.ucsf.edu/docs/tfvm0000

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6. Budget Plan:

Salaries	$ 50,000.00
Expendable Supplies	10,000.00
Permanent Equipment	5,000.00
Overhead	10,000.00
Other	1,000.00
Total	$ 76,000.00

7. Anticipated Duration of Work: Twelve months.

8. Facilities and Staff Available: A pentobarbital is available on hand-size panels of [illegible] and a psychiatric social worker.

9. Additional Requirements: It is anticipated that there will be no additional requirements.

10. Additional information (including relation of work to other projects and other sources of support):

1003541798

Signature: Richard C. Proctor, M.D.
Director of Project

Business Officer of the Institution

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